7th International Symposium on Grapevine Physiology and Biotechnology

ABSTRACTS

June 21-25, 2004
University of California, Davis
Davis, CA

DEPT. OF VITICULTURE & ENOLOGY UNIVERSITY OF CALIFORNIA, DAVIS

INTERNATIONAL SOCIETY OF HORTICULTURAL SCIENCE

ISHS

L'OFFICE INTERNATIONAL DE LA VIGNE ET DU VIN

Tentative Program

Date	Session #	Topic	Abstract #
6/21		Welcome (8:30 am)	
	1	International Grape Genome Program	1 - 5
	2	Genetics/Biotechnology	6 - 10
	3	General Physiology	11 - 17
	4	General Physiology	18 - 22
6/22	5	Source/Sink Relationships	23 - 29
	6	Berry Growth/Metabolism	30 - 33
	7	Water Relations/Physiology	34 - 41
	8	Water Relations/Physiology	42 - 48
	Grape Transformation Concurrent Sessions		49 - 64
6/23	9	Genetics/Biotechnology	65 - 70
	10	General Physiology	71 - 74
	Field Trip to Napa Valley		
6/24	11	General Physiology	75 - 81
	12	Genetics/Biotechnology	82 - 86
	13	Irrigation Research	87 - 92
		Poster Presentations	108 - 140
6/25	14	Grapevine Water Relations	93 - 95
	15	Genetics/Biotechnology	96 - 103
	16	Climate Change & Viticulture	104 - 107

THE INTERNATIONAL GRAPE GENOME PROGRAM

Mark R. Thomas

CSIRO Division of Plant Industry, PO Box 350, Glen Osmond, SA 5064, Australia and CRC for Viticulture, PO Box 154, Glen Osmond, SA, 5064, Australia

The International Grape Genome Program (IGGP) has the ambitious goal of developing a detailed understanding of the grapevine genome and assigning functions and traits to specific genes. Given the magnitude of the task, and in order to ensure rapid scientific advances without unnecessary duplication of effort, cooperation of all nations and scientists involved in grapevine research is encouraged. To facilitate this, the IGGP has adopted a public model of data sharing through the use of public databases (eg, NCBI) and open communication to promote collaborative interactions at the scientific level. The complete analysis of the grapevine genome is being approached through a number of complementary strategies; genetic mapping, physical mapping, EST sequencing, transcriptome analysis, BAC sequencing, bioinformatics and functional analysis. Enormous progress has been made since the formation of the IGGP in 2002 but there are many challenges to overcome if the goal is to be achieved. The willingness and involvement of the international grape community will largely determine the success of the initiative. Outcomes of the program will not only be new scientific knowledge but the future application of this knowledge to industry problems resulting in superior varieties with improved berry quality and disease resistance and better vineyard management leading to low input sustainable viticulture. The IGGP white paper can be found at http://www.vitaceae.org.

AN UPDATE ON CHARACTERIZATION OF THE GRAPE TRANSCRIPTOME THROUGH EST SEQUENCING, TRANSCRIPTIONAL PROFILING AND BIOINFORMATICS

Francisco Goes da Silva[a], Alberto Iandolino[b], HyunJu Lim[a], HongKyu Choi[a], JongMin Baek[c], Anna Leslie[c], Jane Xu[c], Douglas Cook[a,c], Marlene C. Bohlman[d], Fadi Alkayal[d], Mary Ann Cushman[d], Rubi Figueroa-Paredes[d], Elizabeth Tattersall[d], Kitty Spreeman[d], Elif Kabuloglu[d], Chunhong Mao[e], Grant R. Cramer[d], and John C. Cushman[d]

[a]*Department of Plant Pathology, University of California, Davis, CA, 95616*
[b]*Department of Viticulture and Enology, University of California, Davis, CA, 95616*
[c]*CA&ES Genomics Facility, University of California, Davis, CA, 95616*
[d]*Department of Biochemistry, University of Nevada, Reno, NV 89557*
[e]*Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061-0477*

Progress in grape genomics research has been rapid since the initial grape genomics meeting at the University of California, Davis in May 2001. The primary evidence of this is an increase in the number of expressed sequence tags (ESTs) at the National Center for Biotechnology Information (NCBI) database (dbEST), from about 200 in 2001 to over 145,000 sequences as of January 2004. The majority of these public domain sequences were contributed from the UC Davis and UN Reno groups, with funding from the CDFA/ARS, USDA Viticultural Consortium, American Vineyard Foundation and NSF. The availability of a deep EST collection provides an initial assessment of the diversity of grape genes and it will enable a range of functional genomics studies in *Vitis vinifera* and related species, in particular high-throughput gene expression by means of oligonucleotide microarrays. The generation of EST sequences is accompanied by three public bioinformatic efforts (http://cgf.ucdavis.edu; http://www.vbi.vt.edu/~estap/; http://www.tigr.org/tdb/tgi/vvgi) that aim to organize and annotate the grape uni-gene set, and to extract valuable attributes from the data such as relationships to characterized genes in other species and provide a methodological description of genotype diversity (e.g., simple sequence repeat motifs and single nucleotide polymorphisms). The current grape uni-gene set includes approximately 23K *Vitis vinifera* contigs/singletons and approximately 6K contigs/singletons from other *Vitis* species. Statistical analysis and 2-D hierarchical clustering has identified a large number of genes in *V. vinifera* that are differentially regulated during development, or in response to biotic or abiotic stress. The data also provide a first glimpse of transcriptional networks, where correlation analysis is used to evaluate patterns of gene expression within characterized metabolic pathways. In March 2004, a public Affymetrix microarray will be available, composed of ~11,000 distinct probe sets and surveying an estimated 30% of the grape transcriptome. Microarray research in grape should contribute in important and novel ways to the understanding of fundamental aspects of grape biology, such as transcriptional responses correlated with biotic (e.g., pathogen) and abiotic stress (e.g., drought, temperature and salt), gene expression during fruit development, and transcriptional responses to particular viticultural practices. The outcome of such studies will likely impact grape improvement in two ways: first, it will yield marker genes (for example, those that are correlated with particular physiological stresses) that can be used to establish diagnostic assays in the field, and second, it will identify candidate genes that may contribute to the agronomic properties of grape, including disease resistance and quality.

PROGRESS OF THE "BAC LIBRARIES, PHYSICAL MAPS AND GENOME SEQUENCING" WORKING GROUP.

A-F Adam-Blondon

URGV, 2 rue Caston Crémieux CP5708, 91057 Evry Cedex – France

The objectives of this working group are (1) facilitate the construction of a gene map, (2) facilitate the assembly and the finishing of the genome sequence, (3) facilitate the cloning of important genes. To meet these objectives, two reference BAC library made from Cabernet Sauvignon (one by URGV, France and one by Gallo SA) were chosen by the IGGP and provided as public resources. Several approaches are developed:

- 788 STS have been anchored by PCR on pools : genetically mapped markers (233) and ESTs (555). The pools are available on demand and have already been distributed to several groups.
- The two extremities of all the BACs from the URGV library have been sequenced (78600 sequences) by Génoscope (France).
- The fingerprinting of the library is starting using an automated process described by Luo et al (Genomics 2003, 82 : 378-389) and adapted to grape by Moroldo et al (session 2).

In parallel, 4 BACs have been sequenced to assess the nucleotide divergence between the two haplotypes present in the Cabernet Sauvignon genome in two different regions. This will help to define a strategy for the sequencing of the whole genome in collaboration with the Génoscope (France).

PROGRESS IN REFERENCE GENETIC MAP AND REFERENCE POPULATION FOR MAPPING

M. Stella Grando[a] and Agnès Doligez[b]

[a] *Istituto Agrario, San Michele all'Adige, Italy*
[b] *INRA, Montpellier, France*

Several genetic maps of grape presently available were generated with different purposes. In order to harmonize linkage groups resulting from individual mapping projects and provide a resource for physical mapping, the framework linkage map of *Vitis vinifera* cvs. Riesling x Cabernet Sauvignon (Riaz et al. 2003) has been adopted as a reference map for the International Grape Genome Program. This molecular tool is mainly based on SSRs developed in the past five years by the grape genetics community within the Vitis Microsatellite Consortium. Microsatellite markers allow to combine different maps and now an effort is in progress to construct a consensus map exploiting segregating data sets from several experiments. The F_1 progeny of Riesling x Cabernet Sauvignon was also established as the reference progeny for the construction of a high density map to enable fine scale localization and identification of genes responsible for traits of agronomic importance. Responsibility for an expanded Riesling x Cabernet Sauvignon population is planned to be shared by several institutions in the United States, Europe and Australia.

PROGRESS IN BIOINFORMATICS – THE CHALLENGE OF INTEGRATING TRANSCRIPTOMIC, PROTEOMIC & METABOLOMIC INFORMATION

Grant R. Cramer[a], John C. Cushman[a], David A. Schooley[a], David Quilici[a], Delphine Vincent[a], Marlene C. Bohlman[a], Karen Schlauch[b], and Pedro Mendes[c]

[a]*Department of Biochemistry, University of Nevada, Reno, NV 89557*
[b]*Center for Biomedical Genomics and and Informatics, George Mason University, 10900 University Blvd, MS 5E3, Manassas, VA 20110*
[c]*Virginia Bioinformatics Institute, 1750 Kraft Drive, Suite 110, Corporate Research Center, Virginia Tech., Blacksburg, VA 24061*

Regulated-deficit irrigation has been used successfully to grow grapes with less water, an important feature in arid regions like Nevada. An added benefit of water-deficit stress is that it affects important aroma, flavor and color constituents in wine grape berries and wine subsequently made from those berries. These changes can be associated with improved wine quality and human health benefits. We have initiated an integrative and quantitative analysis of mRNA, protein, and metabolite changes following abiotic stress imposition in an effort to enhance production efficiency under stress conditions and to understand the plant-derived contribution to improved wine characteristics. In collaboration with the International Grape Genomics Program community (www.vitaceae.org), we have developed the first Affymetrix GeneChip for *Vitis vinifera* and related hybrids containing probe sets for approximately 20,000 genes, in order to conduct large-scale mRNA expression profiling. In addition, we are using two-dimensional polyacrylamide gel electrophoresis coupled with tandem mass spectrometry, and gas chromatography-mass spectrometry to conduct parallel, large-scale protein and metabolite profiles. Custom bioinformatic resources are being developed to integrate experimental results and information about mRNA, protein, and metabolite level changes that arise in response to abiotic stress exposure. Results from global mRNA expression, protein and metabolite profiling from abiotically-stressed leaves and berries from both long-term and short-stress experiments will be summarized. These analyses will be used to elucidate the identification of specific metabolic pathways that are perturbed by stress treatments. The comprehensive data sets generated by this project will be critical to our ability to consider engineering strategies for improving wine quality in the future.

CONSTRUCTION OF A CONTIG-BASED PHYSICAL MAP OF GRAPE USING FLUORESCENT FINGERPRINTING TECHNOLOGY

M. Moroldo[a,b], S. Scalabrin[a], G. Prete[a], N. Felice[a], R. Marconi[a,b], G. Faes[a,b], R. Velasco[b], and M. Morgante[a]

[a]*Dipt. Scienze Agrarie ed Ambientali, Universita' di Udine, via delle Scienze 208, 33100 Udine, Italy*
[a]*Istituto Agrario San Michele all'Adige, via Mach 1, 38010, San Michele, Trento, Italy*

Many relevant traits are controlled by unknown genes that can be genetically mapped but not easily identified. If their function is unknown, as for most traits of agronomic importance, traditional gene isolation strategies cannot be used. A positional cloning approach is then the only option left. The availability of a physical map that is tightly linked to the genetic map could greatly facilitate positional cloning in grape, once the genetic mapping is performed. A physical contig map would also serve as a prerequisite for any future genome sequencing project. We have made significant progress towards the construction of a whole-genome physical map of grape using a fluorescent fingerprinting approach of a 10X grape BAC library (45000 clones, average insert size 120 kb) from Pinot Noir. DNA is being isolated from each of the BAC clones in a 96-well plate format, digested using restriction enzymes and run out on a ABI3730 DNA sequencer after fluorescent labelling to produce a fingerprint of each individual BAC clone. We have adapted to grape a fingerprinting technology developed by Luo et al. (Genomics, 2003, 82:378-389) for wheat and rice using 5 fluorescent dyes and 5 restriction enzymes. Data have been collected by the ABI GeneMapper software and processed using the software package Genoprofiler and PERL scripts to distinguish peaks corresponding to restriction fragments from peaks generated by background noise. As grape is a highly heterozygous species, we are evaluating the impact on the fingerprints and on the map assembly of allelic differences between the maternally and paternally inherited chromosomes. The accuracy of the contigs is being checked by deriving genetic markers from the contigs and placing them on a genetic map. The process for high-throughput fingerprinting, map assembly and evaluation will be described.

EST DATABASE AND GENE EXPRESSION STUDIES OF ABIOTICALLY STRESSED GRAPEVINE *VITIS VINIFERA* L.

Marlene C. Bohlman[a], Ali Ergul[b], Elizabeth A.R. Tattersall[a], Richard L. Tillett[a], Rubi Figueroa-Paredes[a], Elif Kabuloglu[a], Mary Ann Cushman[a], Kitty L. Spreeman[a], Karen Schlauch[c], Pedro Mendes[d], Grant R. Cramer[a], and John C. Cushman[a]

[a]*Department of Biochemistry, University of Nevada, Reno, NV 89557*
[b]*Institute of Biotechnology, University of Ankara, 06500, Besevler-Ankara/Turkey*
[c]*Virginia Bioinformatics Institute, 1750 Kraft Drive, Suite 1100 Corporate Research Center - Virginia Tech, Blacksburg, VA 24061*
[d]*Center for Biomedical Genomics and Informatics, George Mason University, 10900 University Blvd, MS 4E3 Manassas, VA 20110*

Grapevines exposed to abiotic stress such as drought, salinity and cold produce berries with altered metabolite and glucan composition with little loss in fruit production. These changes are associated with improved wine quality and human health benefits. Regulated deficit irrigation has been used successfully to grow grapes with less water, an important feature in arid regions like Nevada. As the first step of an integrated functional genomics approach to understand how changes in gene expression impact metabolite changes that improve color, aroma, and flavor components of berries and wine, we have constructed five cDNA libraries from mRNA isolated from leaves, berries, and roots of *Vitis vinifera* L. cv. Chardonnay exposed to cold, heat, salt, water deficit, and low-oxygen stress treatments. An initial analysis of more than 52,000 ESTs generated from cDNA clones of these libraries revealed that ~53% of ESTs were novel or shared significant similarity with genes of unknown function. Overall, approximately 14% of ESTs were related to disease/defense or abiotic stress adaptation. The leaf library contained significantly more photosynthesis-related genes than either berry or root libraries, whereas the berry library had significantly more ESTs with secondary metabolism, fruit ripening and flowering related genes than the leaf or root libraries. Additional ESTs have been generated from six water deficit cDNA libraries prepared from mRNA isolated from leaves, berries, and roots of *Vitis vinifera* L. cv. Cabernet Sauvignon to complement the existing EST data set. A unigene set has been assembled and used for the construction of an Affymetrix-based oligonucleotide chip in collaboration with the International Grape Genomics Program (www.vitaceae.org). This Vitis GeneChip contains probe sets for approximately 20,000 genes and is expected to become available to the public in February 2004. We will present mRNA expression profiles from *Vitis vinifera* L. cv. Cabernet Sauvignon subjected to long-term water deficit and salt stress.

GENE EXPRESSION PROFILES IN RESPONSE TO NITROGEN AND CARBON NUTRITION IN *VITIS VINIFERA* L.

Hugues Barbier[a], Jean-Pierre Gaudillere[a] and Christophe Rothan[b]

[a]*Ecophysiologie et Agronomie Viticole, UMR Œnologie Ampélologie, INRA, BP 81, 33883 Villenave d'Ornon, France hbarbier@bordeaux.inra.fr*
[b]*UMR Physiologie et Biotechnologie végétale, INRA, BP 81, 33883 Villenave d'Ornon, France*

Grapevines (Carbenet Sauvignon) were grown under full light and high nitrogen supply (control: NC) and low nitrogen supply (N starved: NS) from fruit set to maturity. Six SSH Banks have been produced from mRNA extracted from roots, leaves and berries (3 NC and 3 NS banks from roots, leaves and berries). A mean of 5000 clones of each bank have been spotted on a nylon Macro-arrays. Analysis of differentially expressed sequence tags revealed grapevine markers for nitrogen and carbon starvation.

CONTROL OF EXPRESSION OF A RIPENING-RELATED GENE IN BERRIES AND SUSPENSION CELLS OF CABERNET SAUVIGNON

Catherine Tesniere[a], Clotilde Verries[a], Martine Pradal[a], Asraf El-Kereamy[b], Laurent Torregrosa[c], Philippe Chatelet[c], Christian Chervin[b]

[a]*UMR 1083, Sciences Pour l'Oenologie, INRA/Agro-M, 2 Place Viala, 34060 Montpellier CEDEX 01, France*
[b]*UMR 990, Génomique et Biotechnologie des Fruits, INRA Toulouse , BP 107, 31326 Castanet, France*
[c]*UMR 1098, Biologie du Développement des Espèces Pérennes Cultivées, INRA/Agro-M, 2 place Viala, 34060 Montpellier CEDEX 01, France*

Many of the molecular events controlling the ripening process in grape berries remain unknown. However, studies on grape ethylene receptors led us to evaluate the role of ethylene in this non-climacteric fruit. The mechanisms that control the up-regulation of *VvADH2* transcription and ADH activity at the inception of fruit ripening were studied. The effects of a specific inhibitor of ethylene receptors and of an ethylene releasing compound are discussed based on experiments on *Vitis vinifera* cv. Cabernet Sauvignon developing berries and suspension cells. We found that the regulatory elements for both ethylene and anaerobiosis responses are present in the *VvADH2* promoter and investigated whether they could participate in the response of the *VvADH2* to hormonal and hypoxic treatments. The results indicate that the ethylene transduction pathway and anaerobic stress could be involved in regulating *VvADH2* expression. These data provide new evidence in the control of gene expression of a ripening-related gene in a non-climacteric fruit.

GENE EXPRESSION PROFILING DURING GRAPE LEAF DEVELOPMENT AND SENESCENCE BY HIGH DENSITY FILTERS

Claudio Moser[a], Massimo Pindo[a], Enrico Blanzieri[b], Massimo Bertamini[a], Cinzia Segala[a], Namachevayam Neduchenziam[a], Paolo Fontana[a] and Riccardo Velasco[a]

[a]*Istituto Agrario San Michele all'Adige, San Michele a/A (TN), ITALY*
[b]*Dipartimento di Informatica, Università di Trento, ITALY*

Leaf development and senescence are biological processes under tight genetic control and characterized by an increase in the photosynthetic rates during leaf expansion and a decline during leaf senescence. To obtain high quality grape berries it is important that in the ripening stage the plant canopy does not lose photosynthetic capacity. Vineyard management practices such as pruning or fertilization can slow down leaf aging but often this will not entirely solve the problem. High-throughput sequencing of expressed sequence tags (ESTs) and gene expression analysis, on a genome-wide scale, have opened new possibilities to shed light on complex biological questions like leaf senescence. Understanding the molecular biology governing the physiology of such processes may help in maintaining the efficiency of the plant for a greater duration of the season. Three years ago we initiated the construction and characterization of cDNA libraries from different grape tissues and the collection of a large number of ESTs. The sequence information has been processed via bioinformatics tools in order to estimate redundancy and to assign a putative function to each clone on homology base. A subset of the cDNA clones from leaf, shoot and inflorescence (ca. 2300 unigenes) have been spotted on high-density nylon filters and probed with RNA isolated from 'Pinot noir' leaves at 5 different developmental stages. Significant differential expression between the experimental conditions has been assessed by the use of paired t-test on log transformed data. The study of gene expression data has mainly focused on genes involved in specific metabolic functions and the results have been interpreted in the light of physiological measurements like pigments changes, electron transport activities and total soluble proteins content. Preliminary results identified groups of genes that are specifically up-regulated during leaf development and senescence.

THE INFLUENCE OF CHILLING ACCUMULATION ON BUD DORMANCY OF CUTTINGS OF SANGIOVESE AND MONTEPULCIANO GRAPEVINES (*VITIS VINIFERA* L.)

Paolo Sabbatini[a], Davide Neri[a], Franco Zucconi[a], Oriana Silvestroni[b] and Elisa Manni[b]

[a]*Dipartimento di Energetica, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy*
[b]*Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Via Brecce Bianche, 60131, Ancona, Italy*

Existing difficulties in the control of bud dormancy partially derive from a lack of knowledge of the physiological factors involved in its onset. The problem is rendered more complex by the fact that dormancy varies both between shoots, depending on vascular transport and growth habits, and along the shoot, depending on the bud position and time of formation. The objective of this work was to analyze the dynamics of bud dormancy in relation to the chilling accumulation during the winter month. The trial was carried out in a 7-year-old vineyard, located in Central Italy (Castelferretti, Ancona, 43°40'N), on a South-exposed hillside (110 m). Sangiovese and Montepulciano vines, grafted on Kober 5BB rootstock, were spaced 1.0 m apart along the rows, and 2.5 m between the North-South oriented rows. Budbreak was analyzed using binodal cuttings, since the inhibition of cell elongation of primary meristem, under favorable conditions, is considered an index that represents dormancy and its intensity. Samples of one-year-old shoots were collected at four times, from November to February. Dormancy was estimated using "one-node-cuttings" and the number of buds that broke was recorded during a 20 day incubation in a growth chamber (24-hr light -120 µmol $m^{-2}s^{-1}$, 20±2 C° and 90% RH). The study of such dormancy shows an own dynamics, decreasing with time and along the shoot axis with a minimum at the apex. It also shows the existence of oscillations in time along the axes, an event that also interacts with cold temperatures during spring growth.

GENETIC SEGREGATION FOR INDICATORS OF PHOTOPERIOD CONTROL OF DORMANCY INDUCTION IN *VITIS* SPECIES

A. Fennell[a], K. Mathiason[a], J. Luby[b]

[a]*Horticulture, Forestry, Landscape & Parks, Northern Plains Biostress Laboratory, South Dakota State University, Brookings, SD, USA*
[b]*Dept. of Horticultural Science, University of Minnesota, 1970 Folwell Ave, St. Paul, MN, USA*

The timing of endodormancy induction and release is important in the economic production of grapes. Overwintering grape buds develop in the leaf axil and are paradormant during the growing season. Endodormancy is induced in the bud by a decreasing photoperiod and/or low temperature during autumn. In grapes there is considerable variation in response to photoperiod, with some genotypes becoming endodormant in response to a decreasing photoperiod (SD) and others requiring low temperature to induce dormancy. A comprehensive understanding of the biological mechanisms involved in bud dormancy is needed to promote advances in selection and breeding as well as developing improved cultural practices for dormancy management in existing cultivars. We have developed a genetic model system with variation in endodormancy initiation and chilling requirement for investigating the mechanisms regulating dormancy induction and release. This system includes two parents: a photoperiod responsive (dormancy induction), cold hardy genotype of *V. riparia*, and the photoperiod nonresponsive, less cold hardy *V.* spp. 'Seyval Blanc' cultivar, their F_1, and F_2 and BC_1 populations. Four morphological traits (shoot elongation, shoot tip abscission, summer lateral emergence and periderm development) and one molecular trait (SD495) have been identified in controlled environment studies as potential selection markers for photoperiod responsiveness and early dormancy induction in *Vitis*. In a replicated field planting of the F_2 and BC_1 populations summer lateral emergence was the most useful for determining photoperiod induced endodormancy. Analysis of this trait indicates that the F_2 population is segregating at about 3:1 and the backcross population at 1:1 for photoperiod responsiveness. SD495 is expressed in *V. riparia* and the F_1 under SD induction of dormancy. SD495 is not expressed in Seyval Blanc nor is endodormancy induced under SD. This genetic system provides a powerful tool for identifying and characterizing other markers for dormancy induction and release in *Vitis*.

FACTORS INFLUENCING PRIMARY BUD NECROSIS (PBN) IN AUSTRALIAN VINEYARDS

C. Collins and B. Rawnsley

South Australian Research and Development, Institute (SARDI), GPO Box 397, Adelaide, South Australia, Australia, 5001

Grapevine buds contain three or more axillary buds with the primary bud producing the fruiting shoots for the coming growing season. Primary bud necrosis (PBN) is a physiological disorder resulting in the death of the primary bud. PBN has been associated with shoot vigour, water stress, shade, plant growth regulators and bud carbohydrates. Microscopic bud dissection used to assess bud fruitfulness and predict potential yield in vineyards has highlighted the incidence of PBN. The highest incidence of PBN in Australia has been found in the cultivar Shiraz. Buds dissected during the 2002/2003 growing season revealed that PBN in Shiraz occurred around flowering, which coincided with the rapid period of shoot growth during spring and bud differentiation. Past research indicated PBN occurred until bud dormancy in summer, however, our findings indicate PBN levels increased up until autumn. Shoot vigour was also assessed and unlike previous research no correlation was found between PBN and excessive shoot vigour. These studies are continuing along with the timing and incidence of PBN in a number of different wine grape cultivars in Australia during the 2003/2004 growing season. Appropriate management strategies, such as modifying pruning levels, are being investigated to minimize the incidence of PBN in susceptible vineyards.

POSSIBLE INVOLVEMENT OF OXIDATIVE STRESS IN BREAKING GRAPE BUD DORMANCY INDUCED BY DIFFERENT STRESS AGENTS

Etti Or[a], Pang Chechoon[a], Halali Tamar[a], Battikoff Tamar[a], Aliza Ogredovitch[a], Omer Krain[a], David Galbraith[b] and Jaganatha Venkateswari

[a]*Department of Tree Breeding, Institute of Horticulture, Agricultural Research Organization, the Volcani Center, P.O.B. 6, Bet Dagan 50250, Israel*
[b]*Department of Plant Science, University of Arizona, 303 Forbes Building, Tucson AZ 85721 USA*

Although bud dormancy has been the subject of many physiological studies, little is known about the mechanisms of bud dormancy induction and release, or their control. Knowledge based on other plant developmental processes indicates that the dormancy cycle might begin with the perception of an environmental cue by the plant, followed by transduction of this signal via a cascade of events up to the stage where it induces repression of bud meristematic activity. Release from endodormancy should depend on a similar cascade that develops upon exposure to chilling temperatures or following the application of alternative means to break dormancy. Alterations in gene expression during early stages of dormancy release in grapevine buds were analyzed, following several treatments that led to budbreak. This kind of analysis allows us to identify gene products that may mediate the signal transduction of a dormancy-release signal, or derepression of meristematic activity. It also facilitates analysis of the degree of similarity between the expression programs induced by different dormancy-breaking agents. Searching for genes showing identical changes in the expression pattern, following the application of different exogenous signals, may allow the identification of key pathways and genes that are essential for dormancy release, independent of the primary artificial signal inducing the process. Here we describe the temporal reduction in the levels of catalase and dehydrin like transcripts following induction of dormancy release by various treatments and the parallel induction of ADH, PDC, GDBrPK, GR and APX transcript levels. Similarities in the pattern of changes following chemical induction, heat shock and other treatments that lead to induction of dormancy release will be presented and related to actual budbreak. The relevance of the described changes to dormancy release and the possible involvement of oxidative stress will be discussed. Preliminary data from a large scale analysis will be presented.

STUDY OF ONTOGENY AND DEVELOPMENT OF INFLORESCENCE IN PRIMARY BUDS OF SULTANIN GRAPE (*VITIS VINIFERA* L.) BY MEANS OF EPI-ILLUMINATION LIGHT MICROSCOPY

M.B. Hassanpouraghdam, A. Nazemieh, M.R. Dadpour and M. Valizadeh

Department of Horticulture, University of Tabriz, Tabriz, Iran

The main objective of this research was to study the time of initiation and branching pattern of the inflorescence in primary buds (1,2,9 and 10) of Sultanin grape. To study these phenomena we used epi-illumination light microscopy. The buds were collected from field grown vines. After fixation and washing and staining, the buds were dissected and their apexes were photographed with a digital camera. The photographs were processed using Photoshop 5.5 software. The results revealed that inflorescence initiation in Sultanin grape at the Khalat Poshan Research Station (Tabriz) takes place around late June and the inflorescence is initiated as an uncommitted Primordium. It then initiates a bract and bifurcates to form an inner and outer arm. Both of the arms initiate inflorescence branches, first in a decussate pattern, then in a 2/5 phyllotactic spiral. These branches may initiate pairs of lateral primordia until a series of third-or fourth order cymose inflorescence branches are initiated. The time between initiation of two inflorescences was 20 days in a single bud while it was around three days in two alternate buds. In relation to the phenological stage of the vine, initiation of the first inflorescence to this cultivar occured when 16 nodes were present on the shoot. This growth stage takes place around two weeks prior the start of flowering.

AROMATIC POTENTIAL OF GRAPEVINES CULTIVATED IN THE NORTH AND THE SOUTH OF TUNISIA

H. Zemni[a], I. Souid[a], A. Mliki[a], R. Hellali[b] and A. Ghorbel[a]

[a]*Institut National de Recherche Scientifique et Technique (L.P.M.V.) BP 95 Hammam Lif 2050. Tunisia*
[b]*Institut National Agronomique de Tunis (L.A.F.) 43 Av. Charle Nicole 1082 Cité El Mahragen. Tunis. Tunisia zemnih@yahoo.fr*

Organoleptic and aromatic characteristics were evaluated for three Tunisian grapevines (Ahmer Bou Ahmer, Sakasly and Muscat d'Alexandrie) cultivated in the South and North of the country. The southern location was the experimental station of the O.D.R.M. in Rjim Maâtoug, dominated by a Saharian climate. The northern location was at the experimental station of the "Institut National de Recherche Scientifique et Technique " where the climate is semi arid. Quality parameters (bunch and berry weight and seed number) and chemical analysis (pH, Brix degree and density) of freshly-pressed juice were analyzed on 5 kg of fruit of each variety from both sites. Solid phase extraction using Amberlite XAD-2 column was employed to isolate free and bound compounds of aroma. The elution was carried out with selective solvents (pentane dichloromethane 2:1 v:v and ethyl acetate). The bound fraction was enzymatically hydrolysed with the β-glucosidase AR-2000. Both fractions were analysed by Gas Chromatography. The results indicated that the environmental characteristics of the South (extreme temperature and sun exposure) induced the degradation of pomological and physico-chemical quality of the fruit for all varieties. Nevertheless, after aromatic analysis, the levels of free and bound monoterpenols and alcohols were improved in the South for the varieties Ahmer Bou Ahmer and Sakasly but decreased for Muscat d'Alexandrie. The value of the three important free monoterpenols (linalool+nerol+geraniol) in the South was of 372, 334 and 705 $\mu g.l^{-1}$, for Sakasly, Ahmar Bou Ahmar and Muscat d'Alexandrie, respectively. However, when cultivated in the North, these values were 156, 158 and 1417 $\mu g.l^{-1}$, respectively. Linalool was most sensitive to the Southern conditions. Indeed, its rate decreased 2 to 6 times in comparison to the North, whereas, geraniol was always the most important monoterpenol in both regions and for all varieties.

EFFECTS OF HIGH TEMPERATURE ON SANITARY AND MORPHOLOGICAL STATUS OF TUNISIAN GRAPEVINES

A. Ben Salem-Fnayou[a], M. Hanana[a], N. Dubuis[b], A. Mliki[a], P. Gugerli[b] and A. Ghorbel[a]

[a]*Laboratoire de Physiologie Moléculaire de la vigne, Institut National de Recherche Scientifique et Technique, B.P. 95, Hammam-Lif, 2050 Tunisia. e-mail: bensalem_f@yahoo.com a.ghorbel@inrst.rnrt.tn*

[b]*Laboratoire de Virologie, Station Fédérale de Recherches Agronomiques de Changins, Route de Duillier, case postale 254, Nyon 1260 Switzerland*

The adaptive responses of four Tunisian grapevine cultivars were evaluated in the north of Tunisia (semi-arid climatic region) and in the Sahara, characterized by severe environmental conditions (high temperatures and solar radiation). The response of these cultivars was investigated at the ultrastructural and sanitary levels. Scanning electron microscopy of the leaf surfaces and sections from Asli, Razegui and Cardinal revealed clear differences in leaf epidermis shape and thickeness between the North and Sahara regions. The Sahara-cultivated grapevines had thicker abaxial leaf epidermises than those grown in the North. This was confirmed by transmission electron microscopy of leaf sections from the same cultivars. Greater cell wall thickening and wax accumulation on the cuticule was measured on vines grown in the Sahara then from the North. Thus, as temperature increased, more wax accumulated on the cuticle, resulting in the formation of dense crusts and plates, which would be effective barriers to cell and plant dessication. At the sanitary level, the leafroll-infected (GLRaV-3, which is known to be able to withstand heat treatment) cultivar Sakasly was cultivated at the Tunisian Sahara experimental plot. During the first year of cultivation, an Elisa test performed on the infected grapevines were negative. Elisa tests performed monthly the second year of cultivation also found the GLRaV-3 infected grapevines virus-free. This result was further confirmed by RT-PCR performed on the same samples under the same conditions. Considering the high ambient temperatures of this region during the summer, a natural heat-therapy hypothesis is proposed.

LEAF AND FRUIT RESPONSES OF WHITE RIESLING GRAPEVINES TO UV-RADIATION IN THE FIELD

Magali Lafontaine[a], Hans R. Schultz[a,b], Borbala Bálo[c] and Gyula Varadi[d]

[a]*Institut für Weinbau und Rebenzüchtung, Forschungsanstalt, D-65366 Geisenheim*
[b]*Fachhochschule Wiesbaden/Geisenheim, D-65366 Geisenheim, Germany*
[c]*Research Institutes of Viticulture and Enology, Eger, H-3301, Hungary*
[d]*Research Institutes of Viticulture and Enology, H-6000, Kecskemét, H-6000, Hungary*

Changes in the stratospheric ozone concentration causes UV radiation to increase. The response to UV-B radiation at the organ and cellular level is mainly an increase in the formation of UV-absorbing compounds to decrease UV radiation penetration into the tissue. Some key enzymes involved in flavonoid biosynthesis and the phenyl-propanoid pathway have been shown to be up-regulated by UV radiation, as are levels of the key antioxidants, glutathione and ascorbate. However, carotenoid pigment formation and the incorporation of nitrogen into amino acids can be inhibited. Since components such as flavonoids, amino acids and carotenoids are important constituents of grapes and can have a marked effect on flavour development, altered UV radiation can be expected to influence them. We investigated the possible effects of UV radiation under field conditions, by selectively attenuating various portions of the light spectrum with polyester and di- and tri-acetate films. The entire canopy was exposed or parts thereof to these conditions during berry development. We evaluated skin pigment composition using a non-destructive spectro-photometric technique. There was a strong UV-induced shift towards the formation of red and brown pigments without affecting sugar levels. Chlorophyll degradation in the berry skin proceeded faster in the high UV radiation treatments and this was assessed non-destructively by measuring berry fluorescence. Amino acid concentration was reduced under high levels of UV-B radiation and both the total bound glycosidic secondary metabolites and phenolics were increased. There were some effects noticeable on fermentation velocity and the retention of free and bound aromatic components in the wine.

DIFFERENTIALLY DISPLAYED PROTEINS IN GRAPEVINE (*VITIS VINIFERA* L.) TISSUES SUBJECTED TO HERBICIDE STRESS

A. Castro[a], N. Zorn[b], C. Carapito[b], A. VanDorsselaer[b] and C. Clément[a]

[a]*Laboratoire de Stress, Défenses et Reproduction des Plantes, URVVC UPRES EA 2069, Université de Reims Champagne Ardenne, UFR Sciences, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France*

[b]*Laboratoire de Spectrométrie de Masse Bio-organique, ECPM, Bât R5, 67087 Strasbourg, France*

Flumioxazin is a soil-applied, peroxidizing herbicide that is used in vineyards. Recent studies have demonstrated the herbicide also causes water stress, membrane alteration and a significant decrease in the photosynthetic activity of non-target seedlings. A proteomic approach allowed us to examine for the first time the effect of flumioxazin on proteome expression in grapevine plantlets grown *in vitro*. About 2000 spots were found after gel silver staining in all tissues investigated (leaves, shoots and roots), showing reproducible levels within repetitions. Two-dimensional protein profile analysis of about 250 suspect spots revealed up to 30 proteins displaying a consistent differential expression pattern when compared with the controls. The most prominent changes were detected in roots and shoots, 24 and 48 h after the onset of treatments, respectively. Twenty-two proteins were found to be newly synthesized under herbicide stress, while only 3 polypeptides were shown to disappear. In addition, 9 spots were found to present significant quantitative variations, indicating an induction or repression effect following treatments. Most proteins were of low to medium molecular weight and a slightly acidic pI, being consistent with the timing of the plant response. MALDI-TOF analysis of spot helped to identify several induced enzymes, including a phosphoglycerate mutase and UTP-glucose-1P urydiltransferase from roots, a thioredoxin peroxidase from shoots, glyceraldehyde-3P-dehydrogenase from shoots and leaves, and an ATP synthase beta subunit, ascorbate peroxidase and aminomethyltransferase from leaves. The synthesis *de novo* of up to 13 different polypeptides belonging to the PRP-10 class, found in both roots and stems, indicated an essential role for this group of proteins in cellular defense mechanisms against flumioxazin. No significant homology was found for the remaining spots after searching various databases. The data indicate a direct effect by the flumioxazin on grapevine proteome expression.

A NEW APPROACH TO EXPAND THE LIFE TIME OF *UNCINULA NECATOR* GROWING *IN VITRO*

Sara Monteiro[a,b], Regina Freitas[a], Ricardo Ferreira[a,b], Artur Teixeira[a]

[a]*Departamento de Botânica e Engenharia Biológica, Universidade Técnica de Lisboa, Instituto Superior de Agromonia, 1349-017 Lisboa, Portugal*
[b]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Apartado 127, 2780 Oeiras, Portugal*

Uncinula necator (Sch.) Burrill is the obligate fungal pathogen responsible for powdery mildew in grapevine (*Vitis vinifera* L.). *U. necator* spores may be germinated *in vitro* for up to 48 h, which is useful in the laboratory assay of potential fungicides. However, the short-lived spores make it very difficult to conduct long-term studies on this fungus. The great difficulty in extracting proteins from grapevine leaves is well documented. This is certainly due to the presence of phenolic compounds. Recent evidence indicates that the extractability of grapevine leaf proteins is dramatically enhanced when the leaves are infected with, suggesting that this fungus depletes the leaves in phenolic compounds for growth. Therefore, we analysed the presence of several phenolic compounds, such as tannins, and were able to determine that their concentration was lower in infected leaves than in healthy ones. We also noted that in growth media mixed with leaf extract, there was a better and faster spore germination and germ tube growth. In this work, we are trying to identify the most relevant leaf compounds necessary for the development of *U. necator* fungal infection, and use them in *in vitro* media to promote a longer life time for this fungus.

GROWTH AND GAS EXCHANGE OF MICROPROPAGATED GRAPEVINE PLANTS IN RELATION TO THE SUCROSE CONCENTRATION IN THE NUTRIENT MEDIUM

T. Slavtcheva1 and V. Dimitrova

Institute of Viticulture and Enology, Kala tepe 1, 5800 Pleven, Bulgaria

Investigations were carried out on the gas exchange of *in vitro* cultured grapevines of cvs, Dimiat (clone 4/38, 26th subculture) and Italian Riesling (clone 3/47, 25th subculture). The micropropagated plants were obtained from one-node cuttings (single leaves attached). The explants were cultured on modified MS medium with half-strength macrosalts, full-strength microsalts and indole-3-acetic acid (1 mg/L). Two concentrations of sucrose in the nutrient medium were tested: 15 and 30 g/L. Rates of photosynthesis (Pn) and dark respiration (Rd) were measured with an infra-red gas analyzer. Pn was measured at four different light levels: 20, 100, 200 and 400 µmol $m^{-2}s^{-1}$. Three series of measurements of Pn and Rd were carried out : a) on days 10, 9, 8 (1st series) and 3, 2, 1 (2nd series) before transfer from *in vitro* culture ; b) on days 21, 22, 23 (3rd series) after the transfer. Rates of transpiration (E) were determined gravimetrically on days 7 and 1 before transfer from *in vitro* culture and on days 24 and 31 after the transfer. Results showed that the plantlets developed well under the experimental conditions. The plantlets cultured on medium with a sucrose concentration of 30 g/L showed better rooting compared to those cultured with 15 g/L. Leaf area per plant was also positively affected by the higher sucrose content. The photosynthetic rate was strongly influenced by the light conditions with all series. During *in vitro* cultivation (1st and 2nd series), Pn increased significantly with sucrose in the nutrient medium. Greater differences between the variants studied were obtained, however, in the second series. During *ex vitro* cultivation (3rd series), the influence of sucrose on Pn was generally not significant. Dark respiration rates (Rd) of *in vitro* and *ex vitro* plantlets were non-significantly affected by the sucrose concentration. E was only slightly affected by the sucrose in the culture medium. However, E was higher with *in vitro* plantlets compared to that of *ex vitro* ones and decreased significantly with time during their cultivation.

ECOPHYSIOLOGICAL RESPONSE TO FLOOD OF SEVEN GRAPEVINE CULTIVARS

F. de Herralde, R. Savé and C. Biel

Departament de Tecnologia Hortícola. Institut de Recerca i Tecnologia Agroalimentàries (IRTA). Centre de Cabrils. Ctra. de Cabrils s/n. 08348. Cabrils (Barcelona). Spain.

Grapevines growing in the Mediterranean Basin are usually grafted onto drought resistant rootstocks to withstand dry soil conditions and produce quality fruit and wines. However, irregular heavy summer rainfall occurs that can lead to flood events. The effects of short-term flood on growth and physiology of seven grapevine (*Vitis vinifera*) cultivars were studied under semi-controlled conditions. The cultivars were 'Alicante Bouschet', 'Black Grenache', 'Cabernet Sauvignon', 'Parellada', 'Tempranillo', 'Sauvignon Blanc' and 'White Grenache' all grafted onto 110-Richter. Young, potted vines of each cultivar were subjected to two treatments: non-flooded (control) and flooded for seven days in late August 2002. Effects of flooding on water oxygen concentration, shoot elongation, leaf area, leaf water and osmotic potential, gas exchange, chlorophyll content and non-modulated chlorophyll fluorescence were measured. Measurements were taken on days 0, days 4th and 7th of flooding and days 3 and 8 of recovery. Flooding led to a reduction in oxygen concentration in rhizosphere causing reduction in water uptake and resulting in a secondary water stress. 'Sauvignon Blanc' showed the highest reduction in leaf water potential. Photosynthetic rate and stomatal conductance were reduced in all cultivars after 3 days of flooding, but they recovered as soon as the flood was withdrawn. Shoot elongation was clearly reduced with respect to the controls of 'Cabernet Sauvignon', 'Sauvignon Blanc' and 'Tempranillo'. Damage to 'Sauvignon Blanc' was considerable, reducing the exposed leaf area to 83% of the control and of the remaining leaves 84% were damaged. Therefore, 'Sauvignon Blanc' did not recover from the flood event during the period of study. 'Alicante Bouschet' was only minimally affected by the imposed stress.

SOURCE-SINK BALANCE IN GRAPEVINE AS AFFECTED BY TRAINING SYSTEM

Giovan Battista Mattii[a] and Simone Orlandini[b]

[a]*Dipartimento di Ortoflorofrutticoltura, University of Florence, Italy, gbmattii@unifi.it, corresponding Author*
[b]*Dipartimento di Scienze Agronomiche e Gestione del Territorio Agro-Forestale, University of Florence, Italy simone.orlandini@unifi.it*

Vegetative growth, productivity and the physiological behavior of Sangiovese grapevines in a Tuscan vineyard were evaluated as affected by two trellis systems; single curtain and a low double curtain (Lyre trellis). The vines were spur pruned and cordon trained. The planting density was 6667 vines/hectare. Research was carried out in 2003. The two trellising systems were distributed in a randomized block design with 4 replications of 3 rows each. Data were collected from the central row. Light interception, photosynthesis efficiency as well as vegetative and productive characteristics were measured. Light interception readings were collected 10 cm above the ground at every 10 cm of row width. Leaf gas exchange measurements were performed with a CIRAS 1 gas analyzer. Light interception is greater for the Lyre system compared to the single cordon, especially mid-morning, when the sun elevation is higher. No differences were obtained in single leaf photosynthesis measurements between the trellis systems indicating that Lyre had a higher whole plant photosynthesis. Vegetative growth was higher for the Lyre. Lyre system had greater yield due to both a higher cluster number and cluster weight compared to the single curtain. No differences in must sugar content, titratable acidity or anthocyanins were observed. In conclusion, the higher intercepting leaf area of the Lyre trellis can support a higher yield without affecting quality. This is probably due to greater whole canopy photosynthesis of the Lyre compared to the single curtain.

INFLUENCE OF CROP LOAD ON CHAMBOURCIN YIELD, FRUIT QUALITY, AND WINTER HARDINESS UNDER MIDWESTERN UNITED STATES ENVIRONMENTAL CONDITIONS

I. Dami[a]*, D.C. Ferree[a], K.S. Kurtural[b] and B.H. Taylor[b]

[a]*The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691 USA*
[b]*Southern Illinois University, 1205 Lincoln Dr. PSGA 4415, Carbondale, IL 62901 USA*

The effects of various crop levels on vine canopy, yield components, fruit composition and winter hardiness in Chambourcin grapevines were evaluated in Illinois and Ohio for two and four years, respectively. In the Illinois study, treatments consisted of three pruning levels of 15, 20 and 25 nodes retained for each pound (454 g) of dormant prunings and three cluster-thinning levels of 1, 2 and 2+clusters (no thinning) per shoot in year one and 1, 1.2 and 1.5 clusters per shoot in year two. In the Ohio study, treatments consisted of three cluster-thinning levels of 10, 20, and 30 clusters retained per vine. Yield components that were affected are as follows: the number of clusters retained per vine increased, crop weight increased, and cluster weight and berry weight decreased. Fruit composition was affected as follows: as the number of clusters retained per vine increased and the total soluble solid content and pH of the grape juice decreased. Acclimation and winter hardiness were affected as follows: as the number of clusters retained per vine increased, the number of ripe nodes and bud cold hardiness (measured as LT50) decreased. There was little interaction between pruning and cluster thinning in both years of the study in Illinois, and Chambourcin grapevines were most affected by cluster thinning in both states. Optimum crop load of Chambourcin in relation to the growing conditions at both sites will be discussed.

VEGETATIVE AND REPRODUCTIVE GROWTH POTENTIAL OF *VITIS VINIFERA* L. CV. MONTEPULCIANO GRAPEVINES TRAINED TO THE 'TENDONE' SYSTEM

V. Nuzzo[a], P. Giorio[b], A.M. Palese[a], L. Lazzarí[a]

[a]*Dipartimento di Produzione Vegetale. Università degli Studi della Basilicata, Potenza, Italy nuzzo@unibas.it*
[b]*CNR-Istituto Sistemi Agricoli e Forestali del Mediterraneo, Via Patacca, 85 -80056- Ercolano (Napoli) Italy*

A study was conducted to evaluate the vegetative and reproductive capacity of Montepulciano grapevines trained to the Tendone trellis system. The study was conducted in southern Italy (40° 20' N, 16° 48' S) during 2001 and 2002 in a 5 year old vineyard using drip irrigation. Vine density was 1,600 vines per ha. Three crop load treatments were imposed at fruit set: UT – unthinned control with approximately 70 clusters per vine, T50 – in which 50% of the clusters were removed (~ one cluster per shoot) and T75 – in which 75% of the clusters were removed (~ one cluster every two shoots). Vegetative and reproductive growth was evaluated by measuring leaf gas exchange, leaf area development, light interception and shoot and cluster growth. Leaf area development was measured by collecting shoots from mature vines monthly beginning 15 days after budbreak until the end of September. Transmitted photosynthetic photon flux density ($PPFD_t$) was measured below the canopy (0.25 m above the soil surface) with a Ceptometer. Incident PPFD ($PPFD_i$) was measured above the canopy every five minutes. Measurements were taken at solar noon on cloudless days from May to September. Intercepted PPFD ($PPFD_{in}$) was calculated as the difference between $PPFD_i$ and $PPFD_t$ within each grid of a grid system below the vines. Gas exchange was measure with a LI-COR model LI-6400 gas exchange apparatus equipped with a red-light (670 nm) diode source. Measurements were made on both shaded leaves and those exposed to direct sunlight. Preliminary results indicate that vines trained to the Tendone system have a high capacity to produce vegetation and fruit. Intercepted light reached a maximum value of about 80% at maximum LAI. Thinned vines has significantly greater shoot leaf area, LAI and light intercepted compared to the control (no cluster thinning). There were no difference among treatments regarding maximum photosynthetic rate or apparent quantum efficiency.

EFFECTS OF EARLY LEAF REMOVAL ON CLUSTER MORPHOLOGY, SHOOT EFFICIENCY AND GRAPE QUALITY IN *VITIS VINIFERA* L. CULTIVARS

S. Poni[a], F. Bernizzoni[a], and A. Cenni[b]

[a]*Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Piacenza, Italy*
[b]*Corso di Laurea in Viticoltura ed Enologia, University of Bologna, Italy*

The effect of early leaf removal on cluster morphology and grape quality was examined under pot and field conditions on Barbera and Trebbiano cultivars which typically have tight clusters and are susceptible to rot. In the pot study (Barbera), non-defoliated vines (A) were compared to defoliation in basal shoot zone (nodes 1 to 8) performed at 50 and 100% pre-flowering (B,C), post-flowering (D,E) or 50% at both times (F). Treatments A, C, E and F were replicated also in the field using Barbera and Trebbiano. Fruit-set was reduced by all treatments, generating looser clusters than the control. Cluster weight was reduced by (min-max) 29-48% (treatments B, F) in the pot study, by 47-69% (treatments F, C) in the field trial on Barbera and by 44-56% (treatments F, C) in Trebbiano. In all cases cluster size limitation was primarily the outcome of reduced berry number rather than restricted berry growth. Sugar concentration was increased by all defoliation treatments as a result of severely limited yield per shoot and final leaf-to-fruit ratios (shoot basis) which were either similar or higher than those of control vines. Shoot efficiency, evaluated as the amount of sugar produced per unit leaf area, did not differ significantly among treatments in the pot or the Trebbiano field study. It was reduced for all defoliation treatments in the Barbera field study. These preliminary data highlight the potential of early leaf removal for crop control and improvement of cluster traits under medium-to-high vigour sites. Work is in progress to evaluate carry-over effects and feasibility of mechanical application in the vineyard.

MANIPULATION OF PHOTOSYNTHESIS IN GRAPE (*VITIS VINIFERA* CV. 'FLAME') BY THE APPLICATION OF TWO SUCROSE ANALOGS

M.E. Tiznado-Hernández*, A.J. Ojeda-Contreras and A. Gardea-Béjar

**Departamento de Tecnología de Alimentos de Origen Vegetal., Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera a la Victoria km. 0.6 Apartado Postal 1735, Hermosillo, Sonora. 83000, México, Phone: +52-(662) 289-24-00 ext. 346, Fax: +52-(662) 280-04-22 e-mail: tiznado@cascabel.ciad.mx*

The translocation of photosynthates from the leaves to fruits during post-veraison is important for the accumulation of sugars and during post-harvest for the accumulation of carbohydrates in perennial tissues. Two sucrose analogs were applied to grape leaves at post-veraison to determine their effects on leaf photosynthesis. It is hypothesized that the accumulation of sugar in leaves will bring about a reduction in photosynthesis and an increase in the photosynthate translocation rate (PTR) out of the leaves. The experiment was conducted in a commercial *Vitis vinifera* 'Flame' vineyard, located 50 km northwest of Hermosillo, Mexico. The sucrose analogs used in the experiment were palatinose and turanose in a 5 mM solution containing a commercial surfactant. For each treatment, five fully expanded leaves exposed to direct sunlight from five different vines were sprayed until run-off with a hand-held sprayer. Control leaves were treated with distilled water. Data was collected between 9:00 and 11:30 h. Photosynthesis rate (PR), stomatal conductance (gs), photosynthetically active radiation (PAR) and leaf temperature (Lt) were measured 0, 10, 20, 30, 60 and 90 minutes after the treatment with a LICOR-6200 portable photosynthesis system. Visual observations of leaf necrosis or chlorosis was evaluated to determine possible toxicity to the sucrose analogs. Statistical analysis of data was made by variance analysis based on a completely randomized design and means separated using the Student-Newman-Keuls test with a confidence level of 0.05. Leaves treated with sucrose analogs did not show any symptoms of toxicity. There was no difference between treatments in gs, PAR and Lt. The application of turanose did not affect photosynthesis whereas palatinose reduced the PR one hour after application. It is concluded that the application of palatinose can reduce the photosynthesis rate and perhaps increase translocation of photosynthates from grape leaves.

ESTIMATION OF PRIMARY AND SECONDARY LEAF AREA OF A 'TEMPRANILLO' GRAPEVINE SHOOT

C.M. Lopes and P.A. Pinto

Universidade Técnica de Lisboa, Tapada da Ajuda, P-1349-017, Lisboa Codex, Portugal

Empirical models for non-destructive estimation methods of grapevine primary and lateral leaf area of the red variety 'Tempranillo' are presented. For estimating primary shoot leaf area, a model based on two variables is presented: measured shoot length and average primary shoot leaf area (estimated from the area of the biggest and smallest leaves and leaf number). For lateral shoot leaf area a similar model with only two variables is proposed. Shoot length is replaced by the variable lateral medium leaf area (estimated from the area of the biggest and smallest lateral leaves). Both models explain a high proportion of the shoot leaf area variability and have a good predicting capability. Validation with an independent set of data periodically sampled along the season shows that those models could predict using a non-destructive, simple and accurate method, primary and secondary grapevine shoot leaf area.

NUTRIENT STATUS OF RECENTLY GRAFTED PERLETTE GRAPEVINES: EFFECT OF ROOTSTOCK

Mandeep Singh* and J.K. Sharma

Department of Horticulture, Punjab Agricultural University, Ludhiana 141006, Punjab (India)

A study was conducted at the Department of Horticulture, Punjab Agricultural University, Ludhiana during 2001-03 on four rootstocks: St. George (*Vitis rupestris*), C-1613 (*Solonis* X *Othello* 1613), C-1616 (*Solonis* X *Riparia* 1616) and Perlette (*Vitis vinifera*) used for budding operations. The Perlette cultivar used as the scion was budded both in February and September. Highest nitrogen and phosphorus was recorded in the petioles of the scion budded on St. George in the first week of February which might be due to better bud union and favourable temperature for root activity. Potassium content was found to be the highest in the petioles of cuttings. Sodium and chlorine contents were found lowest in the petioles of the scion budded on St. George in the first week of February. However, the sodium content was highest in Perlette budded on the Perlette rootstock in the first week of February. Sulfur was highest in the leaves of cuttings. The plants budded in the last week of February accumulated more sulfur than those budded in first week of February. Perlette budded onto Perlette had the highest sulfur content.

HORMONAL STATUS IN GRAPE BERRIES DURING RIPENING: IMPORTANCE OF CALCIUM AND POLYAMINE AND ABSCISIC ACID BIOSYNTHESIS

L. Geny, C. Deytieux, A. Darrieumerlou and B. Doneche

UMR 12-19 Œnologie-Ampélologie /Faculté d'œnologie, Université Bordeaux 2-INRA-ENITA/351 Cours de la libération, 33405 Talence, FRANCE

Calcium, abscisic acid and polyamines are elements essential to the growth and the development of the higher plants. Polyamines have been implicated in many physiological processes such as cell division and embryogenesis while abscisic acid may be associated with the ripening and senescence processes. Calcium is generally accepted as a mediator of many cell responses. The evolution and the distribution of calcium, abscisic acid and different categories of polyamines were analysed in grape berries during ripening to better understand the role of calcium on polyamine and abscisic acid biosynthesis. Treatments, imposed at stage BBCH 71, with calcium and the plant growth regulators abscisic acid and fusiccocin (which is known to have an abscisic acid antagonist effect in plant tissues) modified the ripening process, hormonal status and calcium levels in the grape berry at maturity. The application of calcium induced a delay in the ripening process and was accompanied by an increase in abscisic acid and a decrease of polyamine levels due to polyamine catabolism. The abscisic acid treatment did not modify the calcium concentration in the berry but delayed the ripening process as found with the exogenous application of calcium. Our work established the relationship between calcium, abscisic acid and polyamines in grape berries and the nature of the process which underlie these interactions.

ETHYLENE IS REQUIRED FOR THE RIPENING OF GRAPE

Christian Chervin[a], Ashraf El-Kereamy[a,b], Jean-Paul Roustan[a], Julien Lamon[a], Alain Latche[a], Mondher Bouzayen[a]

UMR 990, INRA/INP-ENSAT, BP 107, 31326 Castanet, France
[b]Present address: Department of Horticulture, Faculty of Agriculture, Ain Shams University, P.O. Box: 68, Hadayek Shoubra, 11241 Cairo, Egypt

While the grape has been classified as a non-climacteric fruit whose ripening is thought to be ethylene independent, we show here that endogenous ethylene production just before veraison (i.e. inception of ripening) is required for (i) the increase of berry size, (ii) the decrease of berry acidity and (iii) anthocyanin accumulation in ripening berries. Our data also show that peak ethylene production just before veraison is associated with increased accumulation of ACC oxidase mRNAs and enhanced ACC oxidase activity and total ACC accumulation. The application of 1-MCP at various times before and after veraison, indicated that only grapes treated at the time of the ethylene peak inhibited ripening. The expression of some enzymes involved in anthocyanin synthesis can be triggered by exogenous ethylene. The expression of UDP-flavonoid-glycosyl transferase, an enzyme involved in anthocyanin stability, has been shown to be dependent upon endogenous ethylene signals.

TOWARDS AN UNDERSTANDING OF TARTARIC ACID BIOSYNTHESIS IN GRAPEVINE BERRIES

Seth DeBolt and Christopher M Ford

School of Agriculture and Wine, The University of Adelaide, 5005, Australia

Berries of the grapevine are among the few fruits in which the major organic acid accumulating during development is L-tartaric acid. Despite differing from malic acid only by an extra hydroxyl group, tartaric acid biosynthesis occurs via a different metabolic pathway. Earlier research demonstrated that in grapevine berries, ascorbic acid is the precursor for tartrate biosynthesis, preceded by a number of oxidative steps to yield the 4-carbon tartaric acid molecule and a 2-carbon fragment. The identity of the genes and enzymes of tartaric acid biosynthesis remains unknown. Tartaric acid plays a major role in warm-climate winemaking, with thousands of tonnes added to juice and must to control pH and produce desirable organoleptic characteristics in the final wine. It is surprising so little information exists of the processes by which tartaric acid is made in the developing grape berry. We have examined the capacity of individual berries to direct the synthesis of tartaric acid and its known intermediate compounds, using HPLC to separate and quantify products formed following feeding with ascorbic acid and a number of its precursors. Results of these experiments indicate that the biosynthesis of ascorbate in grape berries occurs via at least two pathways and that tartaric acid biosynthesis is intimately associated with the metabolism of ascorbate during berry development. In an effort to calculate the total tartrate composition of berries, crystals (raphides and druses) were isolated from immature berries and their composition determined by TEM and X-ray dispersive analysis. Whole-bunch feeding with radiolabelled ascorbate revealed that translocated substrates could support the biosynthesis of tartaric and oxalic acids in plants. We are currently using molecular and genomic approaches to isolate genes associated with ascorbate and tartrate biosynthesis during berry development.

EVIDENCE FOR CAROTENOID-CLEAVAGE ENZYMES IN GRAPES

P. Fleischmann[a], P. Winterhalter[a], and S.E. Ebeler[b]

[a]*Institut für Lebensmittelchemie, Technische Universität Braunschweig, D-38106 Braunschweig, Germany*
[b]*Department of Viticulture & Enology, University of California, Davis, California, 95616*

Degradation of carotenoid precursors leads to the formation of important flavor compounds, the C13-norisoprenoids, which are found in a variety of plant flowers, leaves, and fruits, including grapes. The mechanisms for the formation of the C13-norisoprenoids are not well-understood. While photochemical degradation is a known pathway, evidence for enzymatic cleavage of carotenoid precursors has recently been shown in a limited number of plant species (e.g., star fruit, tea). In this study we show preliminary evidence for the existence of a carotenoid cleavage enzyme in the leaves of Silvaner and Riesling grape leaf homogenates. The enzyme has an estimated isoelectric point of pH 3-4. Further studies are underway to more fully characterize the enzyme and determine its role in grape flavor development.

XYLEM WATER TRANSPORT INTO BERRIES OF GRAPEVINE (*VITIS VINIFERA* L.) DURING FRUIT DEVELOPMENT

S.D. Tyerman[a], J. Tilbrook[a], C. Pardo[b], L. Kotula[c] and E. Steudle[c]

[a]*University of Adelaide, Wine and Horticulture, PMB#1, Glen Osmond SA 5064 Australia*
[b]*CTVV, University of Talca, University of Talca, Av. Lircay S/M, Talca, Chile*
[c]*Department of Plant Ecology, University of Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth, Germany*

In order to investigate the phenomenon of berry weight loss in post-veraison Shiraz berries the water flow properties through the xylem into the berry was quantitatively measured using the root pressure probe and the hydraulic conductance flow meter. Analogous to root pressure probe experiments, the equilibrium xylem pressure could be measured during berry development as well as the resistance to water flow into single berries. The equilibrium pressures were negative pre-veraison but became close to zero post-veraison despite the high sugar concentrations in the berry. The position of water flow resistances in the berry and pedicel could be identified by progressively cutting back through the berry and pedicel and were compared with that of Chardonnay, which normally does not show berry shrinkage. The position of the highest resistance was in the vicinity of the brush region and the receptacle at the base of the berry. The position differed between the varieties in post-veraison berries. Using the hydraulic conductance flow meter whole bunch hydraulic resistance could be measured and was negatively correlated with the number of berries or the weight of berries. This also enabled a measure of hydraulic resistance per berry that could be compared during development and between varieties and rootstocks.

DIRECT MEASUREMENT OF BERRY TURGOR PRESSURE IN *VITIS VINIFERA*: RELATIONSHIP TO VINE WATER STATUS AND DEFORMABILITY, AND EVOLUTION THROUGHOUT DEVELOPMENT

Tyler R. Thomas, Ken Shackel, and Mark A. Mathews*

Department of Viticulture and Enology, University of California, One Shields Avenue Davis, CA 95616-8749 USA E-mail: mamatthews@ucdavis.edu

Veraison marks the onset of several physiological changes, including resumption of growth, adjustments in the berry water budget, increased deformability, and the onset of sugar accumulation. The pressure microprobe was utilized to determine berry turgor pressure (TP) directly and *in situ*. Berry TP was not significantly different at increasing depths from the berry surface. Berry TP increased as growth slowed in Stage II, then decreased prior to veraison. Analysis of Chardonnay, Cabernet Sauvignon, and Pinot noir revealed that the decline in TP precedes the significant increase in softening and sugar accumulation observed at veraison, but TP remains relatively constant and low (< 0.5 Bars) after the onset of ripening. Berry TP was up to 10 times higher before veraison than after veraison. When irrigation was withheld, TP decreased with vine water status before veraison, but was insensitive to similar decreases in vine water status after veraison. The results indicate that veraison involves loss of TP, and is consistent with the hypothesis that the post-veraison berry is hydraulically isolated from the vine.

IMPACT OF WATER STRESS ON CARBON PARTITIONING WITHIN SHIRAZ GRAPE BERRIES

M. J. Collins[a], E.W.R. (Snow) Barlow[a], G. Kelley[b], S. Fuentes[b], and R. Wood[c]

[a]*Agriculture and Food Systems, Institute of Land and Food Resources, University of Melbourne, Australia email: collmj@unimelb.edu.au*
[b]*Centre for Horticulture and Plant Sciences (CHAPS), University of Western Sydney, Australia*
[c]*Wine Network Consulting Pty Ltd, Hawthorn, Australia*

The influence water stress on the partitioning photosynthate within the berry was investigated in a field experiment incorporating Partial Rootzone Drying (PRD) treatments in Northeastern Victoria. The trial used four treatments to investigate whether the "PRD effect" was a function of the stress signals sent by the drying rootzone or the amount of water applied to the vine (water stress). Vines were watered in blocks under 100% drip, 100% PRD, 50% drip and 50% PRD with eight replicates per treatment. The PRD vines were watered either side of the vine on a 10-day rotation. Photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentration were measured for four to six days at pre-flowering, pre-veraison, veraison, veraison + three weeks and shortly before harvest. Soil moisture and drying patterns, stem water potentials, bud numbers, bunch numbers, berry numbers, trunk diameter and shoot growth measurements were also measured at these times. Sugar accumulation and acid degradation was measured weekly on each treatment from veraison to harvest. The development of anthocyanins in the berries was measured fortnightly on each treatment post-veraison. Yield and berry quality data was also taken. Berry quality and subsequently carbon balance in the bunch was examined using HPLC to measure sugars, acids and anthocyanins. Pre-flowering and pre-veraison there was no significant difference in photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentrations between treatments, despite the 50% PRD and 50% control treatments receiving half the water of the other treatments. Post-veraison and harvest data analysis will occur by March 2004.

CHARACTERIZATION OF BERRY METABOLIC PROFILES IN GRAPEVINES BY NMR ^{1}H

G.E. Pereira[a], G. Hilbert[a], J.-P Gaudillere[a], M. Maucourt[b], A. Moing[b] and D. Rolin[b]

[a]*Ecophysiologie et Agronomie Viticole, UMR Œnologie Ampélologie, INRA, BP 81, 33883 Villenave d'Ornon, France pereira@bordeaux.inra.fr*
[b]*UMR Physiologie et Biotechnologie Végétale, INRA, BP 81, 33883 Villenave d'Ornon, France*

The content of sugars, organic acids, amino acids and phenolics are important compounds for grape quality. The aim of this work was to determine the metabolic profile of skin and pulp tissues of red (Merlot) and white (Sauvignon blanc) varieties of grapes in relation to their growing conditions. Nuclear magnetic resonance allows one to quantify many different compounds once in berry extracts. Grapevines were cultivated in different soil types and climate in 2002 in the Bordeaux region of France. Agronomical data were recorded in the same plots. The ^{1}H NMR spectra from 0 to 8 ppm chemical shift of water soluble extract of pulp and skin compounds were done in 15 minutes. Discriminant analysis were performed on spectra data after segmentation of spectra into 200 domains of 0.04 ppm. Principal component analysis and partial least square analysis of skin spectra significantly discriminated mature berries from the different vineyards. ^{1}H NMR spectra of pulp were less discriminating. The most significant resonances, contributing to discrimination, were attributed to sugars and amino acids. In conclusion, ^{1}H NMR of berry skin extracts discriminates berries from different origins more efficiently than classical biochemical analysis based on sugar, acidity and nitrogen measurements. This technique will improve the study of environmental factors on grape quality.

CHANGES IN LEAF WATER STATUS IN GRAPEVINE GRAFTINGS TREATED WITH GROWTH REGULATORS

Slavica Todic

Faculty of Agriculture, Nemanjina 6, 11081 Zemun, Serbia and Montenegro, Europe

The effect of foliar applications of the plant growth regulators, paclobutrazol (1000 mg/L), chlorcholine chloride (200 mg/L) and gibberellic acid (100 mg/L) on leaf water status in grapevine graftings of Cardinal was investigated. After stratification and waxing, young vines were planted into vegetation pots and grown in a glasshouse. Foliar treatments were applied once, twice or three times during the vegetative period, starting on 25 July and every 15 days thereafter. Values of total water potential (Ψ_L) and of relative water content (RWC) were measured over the same period. Results indicate a tendency of increased Ψ_L values in leaves of plants repeatedly treated with a growth inhibitor paclobutrazol (-1.18 MPa) compared with untreated (-1.36 MPa) as well as plants treated twice with gibberellic acid (-1.37 MPa). RWC in leaves was significantly increased in the second half of the vegetative period when paclobutrazol was applied twice (78%) in comparison with the control (75%). Values of both measured indices indicate a more favourable water status of plants treated during the vegetative period with growth inhibitors compared with untreated vines or vines treated with gibberellic acid.

INVOLVEMENT OF PHOTOSYNTHESIS IN THE ACHIEVEMENT OF REPRODUCTION IN GRAPEVINE

Gaël Lebon[a], Christian Magné[a], Olivier Brun[b] and Christophe Clément[a]

[a]*Laboratoire de Stress, Défenses et Reproduction des Plantes, URVVC UPRES EA 2069, Université de Reims Champagne Ardenne, UFR Sciences, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France*

[b]*Mumm-Perrier-Jouet Vignobles et Recherches, Avenue de Champagne, 51206 Epernay, France*

The carbohydrate physiology of developing inflorescences remains poorly understood in grapevine up to flowering despite the fact this species is affected by excessive floral abscission. Using the sensitive Gewürztraminer (GW) and the non-sensitive Pinot Noir (PN) cultivars, we have shown that the variations of carbohydrate contents in the inflorescences at the crucial steps of reproduction (15+2 days and 15+8 days) are correlated to flower abscission. It is thought that carbohydrates required for floral and fruit development originated from root reserves and leaf photosynthesis. Photosynthates produced within an inflorescence may be also involved in the reproductive process. In this study, we measured CO2 assimilation of inflorescences from their emergence up to fruit set of field grown vines. While chlorophyll amount and quality were identical in both cultivars, net CO2 assimilation (*A*) and stomatal conductance (*gs*) were significantly different between GW and PN. Leaf *A* of GW was higher than PN until to stage 15+2 days and became lower beyond this stage. Photosynthesis of inflorescences was 4-5 fold lower than in leaves. However, GW inflorescence *A* was higher than *A* of PN at stages 15+2 d and 15+8 d but thereafter GW and PN inflorescence *A* decreased to 0 at fruit set. Although leaf *gs* was not different between the two cultivars, PN inflorescence *gs* was higher than that of GW from stage 15+2 days to fruit set. In conclusion, differences in inflorescence *A* and *gs* between the two cultivars occurred during critical phases of reproduction. The differences of photosynthesis within the inflorescences of the two cultivars are of their carbohydrate contents and confirm the correlation between carbohydrate physiology and fruit set in grapevine.

MODELLING WHOLE CANOPY LIGHT INTERCEPTION AND CARBON GAIN OF *VITIS VINIFERA* L. UNDER CONDITIONS OF WATER AND NITROGEN STRESS

A.B. Iandolino[a], R.W. Pearcy[b] and L.E. Williams[a]

[a]*Department of Viticulture and Enology, University of California, Davis, CA 95616*
[b]*Evolution and Ecology Section, Division of Biological Sciences, University of California, Davis, CA 95616*

Modification of the grapevine's canopy, with concomitant changes in the spatial distribution of shoots, leaves and clusters, is extensively used in vineyards throughout the world. Carbon assimilation at the single leaf and canopy levels of grapevines is dependent upon canopy management practices as well as water and N availability. A grapevine model was developed to: a.) parameterize a dynamic model of leaf C assimilation of grapevine subjected to different levels of water and N availability and row orientations, b.) model canopy architecture, c.) model whole vine light interception and C gain and d.) evaluate trade-offs arising from scaling the model up from a single leaf to the whole canopy. Data were collected on Cabernet Sauvignon grapevines grown in Napa Valley. Vines were either irrigated at 100% of estimated ET_c or not irrigated and fertilized with 109 kg N ha^{-1} or not fertilized over two growing seasons. Allometric parameters determining spatial localization and relationships among canopy components (nodes and internodes and leaf petioles and blades) were determined. Azimuths, elevation angles and lengths were assumed to follow a normal distribution. These data sets were interpreted with a three-dimensional plant architecture model, Y-PLANT. Carbon gain was estimated using a sub-routine included in Y-PLANT. The model predicted whole canopy C assimilation values and diurnal trends similar to those previously shown for field-grown grapevines. Greater amounts of water and N availability early in the growing season stimulated additional lateral shoot growth and foliage. As a consequence, the relative effective leaf area and light interception efficiency were dramatically reduced. Whole canopy C assimilation was directly proportional to the leaf surface exposed to direct solar radiation. On average, 15 to 20% of the canopy's leaves accounted for 80 to 90% of the C assimilated by the vine. Gas exchange data derived from single leaves can predict whole canopy C gain if coupled to estimates of the vine's canopy surface exposed to direct light throughout the day.

CARBON ISOTOPE COMPOSITION ($\delta^{13}C$) AND WATER USE EFFICIENCY IN GRAPEVINES GROWING UNDER DEFICIT IRRIGATION

C.R. Souza[a,b], T. Santos[b], J.P. Maroco[c], E. Brea[b], M.L. Rodrigues[b], C. Lopes[b], J.S. Pereira[b], M.M. Chaves[a,b]

[a] *Instituto de Tecnologia Química e Biológica, Laboratório de Ecofisiologia Molecular, 6º Piso – Avenida República – EAN, 3781-901 Oeiras Portugal*
[b] *Instituto Superior de Agronomia, Tapada da Ajuda – 1349-017, Lisboa - Portugal*
[c] *Instituto Superior de Psicologia Aplicada, Rua jardim do Tabaco 34, Lisboa 1149-047*

In recent years, deficit irrigation systems, including Partial Rootzone Drying (PRD), have been proposed as irrigation techniques to improve water use efficiency and standardize grapevine yield and quality. The objective of this study was to evaluate the effects of PRD and other irrigation systems on $\delta^{13}C$ of leaves and grape berries and intrinsic water use efficiency (A/gs). This experiment was conducted in Portugal, during the years 2000, 2001 and 2002. Five-year old vines of the cvs. Moscatel and Castelão, grafted onto 1103 Paulsen were used. The treatments were: non irrigated, but rain fed (NI); partial root zone drying (PRD), where 50% of ET_c was supplied to only one side of the root system, alternating sides every 15 days; deficit irrigation (DI), where 50 % of ET_c was supplied to both sides of the row, and full irrigation (FI), corresponding to 100% ET_c. There were good correlations of $\delta^{13}C$ with A/gs as well as with pre-dawn leaf water potential. PRD and DI vines showed improved water use efficiency as compared to FI vines. The comparisons between A/gs and $\delta^{13}C$ of leaves and grape berries and also the isotopic composition in different parts of berries are discussed.

REFLECTIVE MULCH TO ENHANCE BERRY QUALITY IN ONTARIO WINE GRAPES

Jane Coventry, Helen Fisher, Andrew Reynolds and Judith Strommer

Department of Plant Agriculture, University of Guelph, Guelph ON Canada, N1G 2W1
jcovent@uoguelph.ca, hfisher@uoguelph.ca, jstromme@uoguelph
Cool Climate Oenology and Viticulture Institute, Brock University, St. Catherines ON Canada areynol@spartan.brock.ca

The Niagara wine region of Ontario is at the northern fringe of adaptability for *Vitis vinifra*. In some growing seasons many *V. vinifera* varieties do not reach maturity before they must be harvested. Reflective mulch has the potential to improve berry quality through its effect on the microclimate within the canopy and in particular light penetration into the fruiting zone. A replicated experiment involving four *V. vinifera* wine grape varieties was established in a commercial vineyard in 2003. Cabernet Franc berries were assessed at two-week intervals from cluster closure to harvest while Cabernet Sauvignon, Pinot Noir and Pinot Meunier berries were assessed at harvest only. Mulch enhanced the quantity and quality of light into the fruiting zone, increased temperature over solar noon and moderated soil temperatures. Mulch advanced veraison in Cabernet Franc and in general increased Brix, total phenolics, total flavonols, total anthocyanins and stilbenes in all four varieties at harvest. HPLC data revealed a shift in anthocyanin composition toward the red (cyanidin/peonidin). This shift was more pronounced in the Pinot than in the Cabernet varieties. In conclusion there is potential for reflective mulch to improve berry quality in Niagara grown grapes particularly in a short season. The effect on wine quality is the subject of an associated study. Additional potential benefits to vine health, weed, pest and disease control, and fruitfulness in future seasons need to be more fully explored.

WATER STRESS INCREASES POLYPHENOLIC QUALITY IN MERLOT GRAPES

Enrico Peterlunger, Paolo Sivilotti, and Vittorio Colussi

Dipartimento di Scienze Agrarie e Ambientali, University of Udine, Via delle Scienze 208, I-33100 Udine, Italy

The relationship between the grapevine and its environment is fundamental to obtain high quality grapes which in turn may give rise to high quality wines. Water stress has recently been studied as a tool to improve the quality of grapes, resulting in a better polyphenolic pattern of the berries and wine. To study the effect of controlled water stress on the polyphenolic quality of Merlot grapes, the present research was conducted in the field during 2002, in North-Eastern Italy Friuli plain. Ten-years-old Merlot grapevines (clone R18) grafted onto SO4 in a vineyard with loamy soil and 15% gravel were used. Water stress was imposed from véraison onwards maintaining the vines at a stem water potential of -1.4 MPa. Vegetative growth was reduced as well as berry weight and stem water potential for the stressed vines. Berry composition was affected; sugars were increased in stressed grapes as well as total polyphenols and anthocyanins. Catechin concentration in the berry was higher at maturity. Proanthocyanidins were higher in stressed berry skins, but there was no difference in stressed berry seeds. Sensory evaluation of wines obtained from stressed grapes revealed a better aroma pattern in comparison to the control wines; more astringency, colour and more harmony in structure. The method of extraction of polyphenols proved to be extremely important in defining the concentration of total and extractable polyphenols. Two different methods of extraction (with ethanol and with acidified methanol) gave a better picture of the effects of water shortage on Merlot grapes. Controlled water stress proved to be beneficial on the polyphenolc quality of Merlot grapes and its wine.

USING WHOLE-VINE PHOTOSYNTHESIS TO UNDERSTAND THE EFFECTS OF WATER STRESS ON PREMIUM WINE GRAPES

Jorge Perez Peña[a] and Julie Tarara[b]

[a] *Washington State University, 24106 N. Bunn Road, Prosser, WA 99350 USA*
[b] *USDA-ARS, 24106 N. Bunn Road, Prosser, WA 99350 USA*

A six-chamber, mobile field laboratory was used to measure whole-vine photosynthesis of field-grown, own-rooted, drip irrigated *Vitis vinifera* cv. Cabernet Sauvignon under three regimes of regulated deficit irrigation (RDI): 1) standard RDI (70% of vine evapotranspiration ET_c replaced weekly); 2) early deficit (50% of vine ET replaced weekly between fruit set and veraison); and 3) veraison deficit (50% of vine ET replaced weekly between veraison and harvest). When not under 50% deficit, vines in scenarios #2 and #3 were irrigated according to standard RDI practice. Vines were planted in 1992 in rows oriented N-S, with spacing of 6 feet between vines and 9 feet between rows at Paterson, WA. Whole-vine chambers were deployed for 7 day measurement runs during physiologically important stages: fruit set, pre- and post-veraison, and pre- and post-harvest. Chambers simultaneously measured two vines per treatment, data collected for 48 hours and then the chambers moved to nearby vines until six vines per treatment were sampled. On adjacent vines, single-leaf measurements of photosynthesis were collected at the same time as the whole-vine measurements. Leaf area per vine was estimated at each stage. Large differences were observed in net carbon exchange and in transpiration between vines under the standard RDI practice and those under additional water stress. In the pre-veraison period, 'early deficit' vines fixed up to 40% less carbon during the middle of the day than did vines under standard RDI. A similar reduction was observed in sunlit, single leaves measured independently of the whole-vine chambers. Vines under early deficit transpired up to 62% less than those under standard RDI. Small differences were detected in net carbon exchange before and after harvest, where temperature was lower and day-length was shorter. After harvest all vines were irrigated as the standard practice until leaf fall.

PIERCE'S DISEASE SYMPTOMS: COMPARISON WITH SYMPTOMS OF WATER DEFICIT AND THE IMPACT OF WATER STRESS

E.T. Thorne[a], M.A. Matthews[a], J.F. Stevenson[b], T.L. Rost[b], J.M. Labavitch[c]

[a] *Viticulture and Enology Dept., University of California, Davis, CA 95616*
[b] *Section of Plant Biology, University of California, Davis, CA 95616*
[c] *Pomology Dept., Univ. of California, Davis, CA 95616*

Plant water status has been recognized for decades as a pivotal factor in Pierce's disease (PD), yet relatively little work has been done to elucidate how plant water relations influence disease development. Visual symptoms of Pierce's disease have been attributed to impaired plant water transport caused by the bacterium, *Xylella fastidiosa* (Xf) clogging xylem vessels. However, many of the symptoms of PD are not the same as those characteristic of water stress or drought. To evaluate the impact of vine water status on the development of disease symptoms and determine whether PD symptoms are a direct result of water deficits, grapevines (*Vitis vinifera* cv. "Chardonnay") were inoculated with Xf or deionized water and subjected to three watering regimes, two of which resulted in the plants experiencing water deficits over an extended period of time (drought). Rapid drying was also imposed on non-infected plants to determine if drying rate influenced water stress symptoms. The visual characteristics of drought and rapid water stress were distinctly different. Likewise, vines infected with Xf showed a number of symptoms unique to PD. Water-stressed vines inoculated with Xf displayed more extensive PD symptoms throughout the plant than did well-watered vines; however, drought did not affect the nature of the PD symptoms. These results suggest that the visual symptoms of PD are not due solely to plant water relations, and that other factors are involved in symptom development.

GRAPEVINE DEFICIT IRRIGATION BY PARTIAL ROOTZONE-DRYING MODIFIES CANOPY MICROCLIMATE AND IMPROVES FRUIT QUALITY WITHOUT AFFECTING YIELD

Tiago P. dos Santos[a], Carlos M. Lopes[a]; M. Lucília Rodrigues[a], Claudia R. de Souza[b], Jorge R. Silva[a], João P. Maroco[c], João S. Pereira[a] and M. Manuela Chaves[a,b]

[a]*Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal*
[b]*Laboratório de Ecofisiologia Molecular, Instituto de Tecnologia Química e Biológica, Apartado 127, 2780-901 Oeiras, Portugal*
[c]*Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco, 34, 1149-047 Lisboa, Portugal*

New deficit irrigation techniques allowing better control of vegetative growth has been developed over the last years to maintain yield and high berry quality, while saving water. The aim of this study was to evaluate the effect of different irrigation strategies on vine water status, canopy microclimate, fruit composition and yield of Castelão, a red wine cultivar. In addition to the non irrigated (NI) treatment three irrigation regimes were applied: partial root drying (PRD, 50% of crop evapotranspiration (ET_c) supplied to only one side of the root system while the other one was allowed to dry, alternating sides approximately every 15 days); deficit irrigated (DI, 50% ET_c supplied, half of it to each side of the root system) and full ET_c (FI). Throughout the growing period mean predawn leaf water potential (Ψ_{pd}) values were -0.2 MPa, -0.3 MPa, -0.4 MPa for the FI, PRD and DI vines, respectively, while Ψ_{pd} progressively decreased in NI plants attaining -0.8 MPa at the end of August. PRD resulted in a lower vegetative growth when compared with FI and DI although higher than NI vines. As a consequence PRD vines showed the highest proportion of exposed clusters within the irrigation treatments, which induced a higher berry temperature. Berry skin anthocyanins were higher in NI and PRD treatments than in DI and FI while phenols content was maximal in PRD vines. Irrigation had no significant effect on berry sugar accumulation and led to a higher yield than the non-irrigated treatment; no significant differences were found among the three irrigated treatments. In conclusion, the more open canopy of PRD irrigated vines allowed a better fruit microclimate that induced an improvement in fruit quality as compared with the other irrigation treatments.

USE OF INFRARED THERMOGRAPHY TO ASSESS SPATIAL AND TEMPORAL VARIABILITY OF STOMATAL CONDUCTANCE OF GRAPEVINES UNDER PARTIAL ROOTZONE DRYING. AN IRRIGATION SCHEDULING APPLICATION

S. Fuentes[a], G. Kelley[a], M. Collins[b], G. Rogers[c,d] and J. Conroy[a]

[a]*Centre for Horticulture and Plant Sciences (CHAPS), University of Western Sydney, Australia, e-mail: s.fuentes@uws.edu.au*
[b]*Agriculture and Food Systems, Institute of Land and Food Resources, University of Melbourne, Australia*
[c]*AHR Crop Science, University of Sydney NSW, Australia*
[d]*Faculty of Agriculture, Food and Natural Resources, University of Sydney NSW, Australia*

A thermal imaging technique, to assess spatial and temporal stomatal conductance, was studied in a grapevine partial rootzone drying (PRD) field trial at Benalla Vic., Australia, in the 2003-04 growing season. Images were obtained using an infrared portable camera (Therma CAM PM575, FLIR systems), on the shaded side of the canopy, for more stable conditions of stomatal conductance estimation. Dry and wet reference leaves were used as reference surfaces and to obtain thresholds to eliminate non-leaf material temperatures in the analysis. An index proportional to stomatal conductance was obtained and compared with stomatal conductance as measured by gas exchange (Licor 6400). Stem water potential was also measured to determine the effect of plant water status on reference temperatures. This technique gives a more accurate estimation of actual stomatal conductance of the whole canopy and canopy resistance (inverse value), which could be incorporated in the Penman-Monteith model to estimate real evapotranspiration on grapevines under PRD.

WATER RELATIONS OF FIELD GROWN DRIP IRRIGATED "TEMPRANILLO" GRAPEVINES IN REQUENA, SPAIN

D.S. Intrigliolo[1], D. Pérez[2], J.R. Castel[1]

[1] *Instituto Valenciano de Investigaciones Agrarias, Dept. Recursos Naturales, Apartado Oficial 46113, Moncada (Valencia), Spain*
[2] *Caja Campo, Servicio Agronómico, Avda de Arrabal, 18, 46310 Requena (Valencia); Spain*

A field experiment was conducted during 2003 in a Tempranillo vineyard where aspects of water relations were studied under two contrasting situations: i) drip irrigation at 100% of estimated crop evapotranspiration and ii) rain fed. As expected non-irrigated grapevines had lower leaf and stem water potential and stomatal conductance during most of the season, in reasonable agreement with the evolution of soil water content measured with capacitance probes. All Ψ measuring methods correlated significantly with one another. However, differences between the two groups of plants were clearer for stem water potential determinations carried out early in the morning, as consequence of stomatal closure that occurred at midday. We also checked for the suitability of several indexes calculated from determinations of trunk diameter variations (TDV) as water stress indicators. In general, a very distinct behavior for all the TDV calculated indexes was observed before and after veraison. The evolution of the daily maximum trunk diameter was a good indicator of water stress until veraison, but not afterwards as trunk growth, in both irrigated and rain fed situations, practically ceased by that phenological period. The maximum daily shrinkage (MDS) gave poor indication of the plant water status even when corrected by trunk phloem thickness.

ISOLATION AND EVALUATION OF ANTIFUNGAL GENES FOR USE IN GRAPEVINE BIOTECHNOLOGY

M.A. Vivier[a], A. De Ascensao[a], M. Carstens[a], E. Basson[a], A. de Beer[a], J. Becker[a], and I.S. Pretorius[b]

[a]*Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, Stellenbosch, South Africa*
[b]*Australian Wine Research Institute, Adelaide, Australia*

Several classes of antifungal proteins and their encoding genes have been confirmed experimentally to be valuable in strategies to increase crop plants' resistance against disease. These include hydrolytic enzymes such as glucanases and chitinases, antifungal peptides such as defensins as well as proteins involved in defence signalling. Our approach to improve disease resistance in *Vitis vinifera* L. includes specific target genes involved in different aspects of the resistance response of plants. To this end various genes from a variety of sources have been isolated and evaluated for their *in planta* potential to decrease disease susceptibility. The genes that were evaluated encoded a chitinase and glucanase from the yeast *Saccharomyces cerevisiae*, a defensin from *Heuchera sanguinea*, polygalacturonase-inhibiting proteins (PGIP) from *Phaseolus vulgaris* and *V. vinifera* and a phytoalexin from grapevine. The genes were all overexpressed in plants and the resultant transgenic lines were evaluated for the integration and expression of the transgenes as well as the presence and/or activity of the encoded products. None of the transgenic lines appeared phenotypically different from the untransformed control plants. Transgenic lines were subjected to infection by *Botrytis cinerea* in a detached leaf assay to evaluate the degree of improved disease resistance obtained. The yeast chitinase, antifungal defensin as well as the PGIP from *V. vinifera* showed significant decreases in disease susceptibility, ranging from 40-85%, when compared to the untransformed control plants. The presence and activities of the heterologous proteins in the various transgenic lines could also be correlated to the observed decreased susceptibilities against the fungal pathogen. These results have provided us with the ability to select promising candidate genes and/or combinations of genes for the genetic improvement of *Vitis vinifera* cultivars.

DEFENSE-RELATED CANDIDATE GENES IN *VITIS* SPECIES

Wenping Qiu[a], Hesheng Hou[b] and Laszlo Kovacs[a]

[a]*Department of Fruit Science, Southwest Missouri State University, Mountain Grove, MO 65711*
[b]*Department of Biology, Liaoning Normal University, Dalian, China 116029*

It is becoming evident that grapevine encodes resistance genes and that it employs both hypersensitive response and ontogenic resistance mechanisms to defend itself against attacks by fungal pathogens. Recently, resistance gene analogues, such as genes containing the NBS-LRR domains, have been identified in wild *Vitis* species and found to be tightly linked to a powdery mildew resistance locus in *Muscadinia rotundifolia*. Transcription factors that orchestrate defense responses in other plants have also been identified in grapevine, and their sequences and transcriptional expression levels have been found diverged between disease-resistant and susceptible *Vitis* species. Defense-related genes encoding non-race specific resistance proteins, stilbene synthases, and pathogenesis-related proteins including chitinases, glucanases, and thaumatin-like proteins, have also been isolated from *Vitis*. Genetic marker-based mapping has lead to the identification of several quantitative trait loci (QTL) for resistance to downy mildew, powdery mildew, and black rot. QTL mapping and segregation analysis results also demonstrate that resistance to certain diseases is a monogenic trait, while resistance to others is multigenic. These discoveries suggest the existence of classical defense mechanisms in *Vitis*. Thus, we shall be able to elaborate and design strategies to exploit the resistance candidate genes for improving disease resistance in susceptible elite grape cultivars.

OVEREXPRESSION OF THE *VITIS VINIFERA* L. β–CAROTENE HYDROXYLASE GENE IMPROVES THE PHOTOPROTECTIVE ABILITY OF TOBACCO

Philip R. Young[a], Melané A. Vivier[a], Shang W. Chen[b] and Isak S. Pretorius[c]

[a]*Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, Stellenbosch, South Africa*
[b]*Food Science and Nutritional Engineering College, China Agricultural University, Beijing, Peoples Republic of China*
[c]*Australian Wine Research Institute, Adelaide, Australia*

Every year severe crop reductions are caused by extreme environmental conditions that typically cause oxidative damage in plants. Carotenoids associated with the photosynthetic membranes serve a vital protective function under these conditions, and are proving a promising target for manipulation in order to enhance the plants' inherent stress tolerance. To this end, the cDNA- and genomic copies of the β–carotene hydroxylase (BCH) encoding gene from *Vitis vinifera* has been isolated and characterized. The BCH gene contains six introns and is present as a single-copy gene in the grapevine genome. The BCH gene is expressed at relatively low-levels in leaves, berries, and flowers. A 2.0 kb fragment of the putative BCH promoter showed no transcriptional activity in transient reporter gene assays. *Nicotiana tabacum* was transformed with the BCH-encoding gene from grapevine which was constitutively overexpressed in both the sense and antisense orientation. Under mild photon flux densities (200-400 μmol m^{-2} s^{-1}) the T_1-generation expressing the BCH gene were phenotypically indiscernible from untransformed plants, and showed no differences in either growth rate or total carotenoid content. Under high light intensities (1500-2500 μmol m^{-2} s^{-1}), however, the photosynthetic parameters of the transgenic tobacco lines showed significant differences relative to both the control and antisense plants. Chlorophyll *a* fluorescence assays revealed that the expressed BCH assisted the transgenic plants in coping with high light stress by reducing photodamage to the PSII reaction centre while maintaining a lower D1 protein turnover rate, as well as a higher net assimilation rate. The rate of recovery and relaxation of the active reaction centers of the transformants during the dark phase was higher than that of the control plants. During the illumination phase the transgenic plants displayed slightly elevated xanthophyll pigment pools in leaves and managed to maintain higher stomatal conductance. Cumulatively, these results indicate that the expression of the grapevine BCH functions in transgenic tobacco by enhancing the plants existing photoprotective system under high light stress. This can be accounted for by the availability of additional zeaxanthin in the xanthophyll biosynthetic pathway.

IDENTIFICATION OF STRESS-INDUCIBLE PROMOTERS IN *VITIS VINIFERA*

Richard L. Tillett, Grant R. Cramer, John C. Cushman

University of Nevada-Reno

Improving the water-stress tolerance of grapevines may improve the utility of grapes as an alternative crop in arid agricultural settings. Strategies for engineering improved abiotic stress tolerance using regulon engineering are enhanced through the use of stress-inducible promoters by avoiding the deleterious effects (e.g. stunting) of constitutive expression of stress-adaptive determinants (Kasuga et al 1999). Although a *Vitis vinifera* GeneChip is under development, results from gene expression studies of *V. vinifera* exposed to abiotic stresses are not yet available. However, microarray experiments conducted in *Arabidopsis thaliana* has led to the identification of large numbers of genes that are induced by cold, salt and water-deficit stress (Kreps et al 2002). Using *A. thaliana* genes induced by all three stresses, we have identified 58 candidate *V. vinifera* orthologs in the TIGR Grape Gene Index (http://www.tigr.org/tdb/tgi/vvgi/) that are likely to be induced by these same stresses. Once their expression patterns are confirmed in *V. vinifera*, we will use LM-PCR to isolate and sequence corresponding 5' flanking regions of selected grape genes. Once characterized, these 5' flanking regions will be used for *in vivo* functional testing to confirm that they confer abiotic stress-inducible gene expression. The availability of stress-inducible promoters from *V. vinifera* will permit efficient regulon or signaling pathway engineering strategies using selected stress tolerance determinants from wine grapes.

ISOLATION, CHARACTERISATION AND FUNCTIONAL ANALYSIS OF A POST-VÉRAISON RIPENING-RELATED PROMOTER ELEMENT FROM *VITIS VINIFERA* CV. MERLOT

Anita L. Burger[a], Leonara Watts[b] and Frederik C. Botha[a]

[a]*Institute for Plant Biotechnology, University of Stellenbosch, Stellenbosch, Republic of South Africa. Current Address:Biotechnology division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, Republic of South Africa*
[b]*Biotechnology Division, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa. Current Address: South African Sugar Experiment Station, Private Bag X02, Mount Edgecombe, 4300, Republic of South Africa*

A major stumbling block in the genetic manipulation of grapevine remains the availability of promoter elements to regulate transgene expression specifically in the fruit-tissue of post-véraison berries. This study focuses on the identification of a ripening-related gene specifically expressed in the grape berry during the post-véraison stages of berry ripening, and the isolation and characterisation of a promoter element from this gene. Due to the low genetic transformation efficiency, slow regeneration (about 18 months) and long reproductive life cycle of grapevine, tobacco and strawberry were evaluated as alternative systems to verify the functionality and specificity of this promoter element in stably transformed plants. To visualise its functionality, the 2.3kb promoter element was fused to the green fluorescent protein (GFP) reporter gene. In tobacco, the genetic element was shown to be functional by the abundant level of GFP visualised in the nectary tissue of the developing flower. In strawberry, the genetic element proved to be functional by the visualisation of GFP in the fruit tissue of the ripening berry. These transgenic strawberries represent the first fruit tissue in which a genetic element isolated from grapevine, can be evaluated.

AGROBACTERIUM TRANSFORMATION OF *VITIS* CELL SUSPENSIONS: IMPROVEMENT OF TRANSFORMATION EFFICIENCY AND TRANSGENIC PLANT REGENERATION USING CRYOPRESERVED CELLS

Qiaochun Wang, Ping Li, Nachman Sahar, and Avihai Perl

Department of Fruit Tree Sciences, Agricultural Research Organization, The Volcani Center, P.O.Box 6, Bet Dagan 50250, Israel

Somatic embryogenesis has been shown to be the most frequently regenerative system in transformation studies. The retention of the embryogenic potential of such cultures during long-term maintenance has proved to be difficult. Cryopreservation is considered as an ideal means of avoiding loss of embryogenic potential during repeated subcultures and as a means of preventing the occurrence of somaclonal variation during long-term maintenance of embryogenic cultures. Embryogenic cell suspensions of grapevine rootstocks 110 Ritcher, B41 and four additional commercial cultivars of *Vitis vinifera* were successfully cryopreserved using either the encapsulation-vitrification or the encapsulation-dehydration methods. All cultivars were successfully cryopreserved and subsequently regenerated into plants. Cryopreservation was found to enhance embryogenesis and subsequent plant germination. We assume that those cells that still maintain their morphogenetic potential survive better the freezing and thawing procedures. The efficiency of embryo formation, embryo development, germination and subsequent conversion into normal plants was significantly improved if cryopreserved cells were utilized. Differences in transformation efficiency were observed between cryopreserved and non-cryopreserved cells using transient studies 24 – 48 h following 5 min of co-cultivation. We cannot rule out the possibility that *Agrobacterium* may have some preference to the host cells upon the recognition and attachment stages. Moreover, conversions of transformed cells to embryos and plantlets were also significantly improved when cryopreserved cells were used as the target materials. The presentation will describe key factors affecting tranformation efficiency and methods to overcome bottlenecks in plantlet regeneration and conversion. One of these improvement is the use of a new type of Chitogel to improve embryo germination following transformation.

APPLICATION OF THE BIOLISTIC METHOD FOR GRAPEVINE GENETIC TRANSFORMATION

Julie R. Kikkert, José R. Vidal, and Bruce I. Reisch

Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, 633 W. North Street, Geneva, NY 14456-0462 USA

Biolistics (particle bombardment) is a widely used method for genetic transformation, however, it has been less frequently employed in grapevines compared to *Agrobacterium*. Our laboratory successfully used biolistics to obtain high frequency transformation and regeneration of 'Chancellor'(hybrid) grapevines with reporter genes and *Vitis vinifera* 'Chardonnay' and 'Merlot' with reporter and/or antimicrobial genes (endochitinase and magainin-type). Embryogenic cell suspensions were spread on filter papers (8 cm dia.) and placed on bombardment medium [half-strength MS with 0.125 M mannitol, 0.125 M sorbitol, 3.0% (w/v) sucrose and 0.25% (w/v) Phytagel (Sigma)]. Cells were bombarded with DNA-coated 1.0 µm gold particles using a prototype of the PDS-1000/He device (BioRad Laboratories, Hercules, CA USA) (1,000 psi, 1 cm gap distance, 1 cm target distance). Petri plates were incubated in the dark at 23 ± 1°C for 2 days and the osmotic pressure of the medium was gradually reduced by transfer to embryo induction medium [half-strength MS medium with 3.0% (w/v) sucrose, 0.3% (w/v) activated charcoal and 0.7% (w/v) Bactoagar]. Two days after bombardment with pBI426 (double CaMV 35S promoter, AMV leader sequence, *uidA+nptII* gene fusion, nos terminator), between 1,000 to 8,000 GUS-expressing cell foci per filter paper were obtained. Stable GUS expression declined rapidly over time. For example, in 'Chardonnay' there were 46 + 32 blue spots per filter 95 days post-bombardment. After selection on kanamycin-containing medium, up to 100 putatively transformed embryos of 'Chancellor' were regenerated per plate. However, with 'Chardonnay' and 'Merlot', 3 to 10 putatively transformed embryos were usually obtained per plate. Plants were regenerated from 54% of 'Chardonnay' embryos. The number of gene insertions ranged from 1 to 5 as determined by Southern blot analyses. Transgenes were successfully expressed in most transgenic lines. The advantages and disadvantages of the biolistic method as well as tips for success will be discussed.

GENETIC TRANSFORMATION OF TABLE GRAPE VIA ORGANOGENESIS AND FIELD EVALUATION OF *DEFH9IAAM* TRANSGENIC PLANTS

Bruno Mezzetti[a], Oriana Silvestroni[a], Elisa Costantini[a], Tiziana Pandolfini[b], and Angelo Spena[b]

[a] *Dipartimento Scienze Ambientali e delle Produzioni Vegetali, Marche Polytechnic University, Via Brecce Bianche 60100 – Ancona (IT) – (Email:bruno@univpm.it)*
[b] *Dipartimento Scientifico Tecnologico, University of Verona, Strada Le Grazie, 34134 Verona (IT) – (Email:angelo.spena@univr.it)*

An alternative method based on regeneration via organogenesis of table grape (*V. vinifera*) was developed by promoting the formation of meristematic bulk (MB) tissue with a high regenerative capacity, using adventitious shoots as a starting material. The regeneration efficiency of slices prepared from the meristematic bulk was higher than that of either lateral axillary shoots or leaves. These slices were used for *Agrobacterium*-mediated transformation of two table grape cultivars (Silcora and Thompson Seedless) with the parthenocarpic chimeric gene *DefH9-iaaM* (Rotino *et al.*, 1997). The aforementioned chimeric gene has been shown to confer parthenocarpic fruit development to several horticultural species, improving both qualitative and quantitative fruit production of tomato and eggplant (Ficcadenti et al, 1999; Donzella et al., 2000; Pandolfini et al., 2002), strawberry and raspberry (Mezzetti et al., 2002, 2004). After rooting and greenhouse acclimation, transgenic plants were transferred to the field. The expression of *DefH9iaaM* gene was detected in transgenic flower buds of both cultivars which corresponded with an increased auxin content of the *DefH9-iaaM* clones in comparison with the control. After three years of field cultivation, *DefH9-iaaM* grape plants were phenotypically homogeneous and did not show any morphological alterations in vegetative growth. Depending on the variety, the ovule specific expression of the *iaaM* gene influenced shoot fruitfulness, cluster morphology and berry quality, in the third year of production.

INDUCTION OF SILENCING IN TRANSGENIC GRAPEVINE (*VITIS* SP) PLANTS

R. Jardak-Jamoussi[b], R. Ebel[a], C. Dubois[a], T. Manthey[a], A. Bassler[a], A. Mliki, B. Bouamama, T. Wetzel[a], A. Ghorbel[b], G. Krczal[a], G.M. Reustle[a]

[a] *Centrum Gruene Gentechnik, DLR-Rheinpfalz, Breitenweg 71, D-67435 Neustadt / W, Germany*
[b] *Institut National de Recherche Scientifique et Technique, Laboratoire de Physiologie Molécuaire de la Vigne, BP 95, Hammam-Lif. 2050, Tunisie.*

The Fanleaf disease, caused by a group of nepoviruses, is a major virus disease in viticulture world wide. The viral infections dramatically decrease the value of an infested vineyard because of the decrease in both the yield and quality of the grapes. Due to a lack of natural genetic resources for virus resistance, suitable for cross breeding programs, a transgenic approach was chosen to develop rootstocks and varieties resistant against the most relevant agents of the Fanleaf disease in German and Tunisian viticulture. Highly conserved sequences of Grapevine Fanleaf Virus (GFLV), Arabis Mosaic Virus (ArMV) and Raspberry Ringspot Virus (RpRSV), were combined with defective interfering (DI)-sequences from a Potyvirus and / or used to clone directed or inverted-repeat constructs. As demonstrated by several authors, this strategy allows the induction of sequence specific RNA-silencing by the expression of aberrant and / or dsRNAs, resulting in resistance against viruses with homologues sequences. For proof of concept the constructs were genetically transferred into tobacco (*N. benthamiana*) by *Agrobacterium* mediated transformation. Selection of transgenic tobacco plants were carried out by using *nptII* (kanamycin resistance) or *bar* (Phosphinotricin resistance) as selectable marker genes. Challenge inoculation with the relevant viruses yielded transgenic lines showing immunity, recovery, retarded infection and susceptibility. Preliminary Northern analysis using a virus-derived sequence as probe, could detect transgene specific siRNA in the resistant lines. To genetically engineer grapevine, embryogenic tissue of rootstocks (SO4, 125-AA, 5C, Binova) and the Tunisian variety Arich were induced from anther cultures and used for *Agrobacterium* (LBA 4404) mediated transformation. After 16 to 20 weeks of selection on phosphinothricin (PPT) containing media, newly generated somatic embryos were harvested and cultivated on PPT free media for regeneration. Transgenic grapevines were regenerated from these embryos and were propagated and maintained *in vitro*. Analysis of the regenerated lines by PCR showed high number of escapes and chimeric character of the plants. Repeated propagation of putative transgenic lines by one node cuttings and PCR analysis of the therefrom obtained plants yielded non-chimeric transgenic grapevines. Molecular analysis showed different copy number in the transgenic lines (1 to 3 copies). Further molecular analysis to confirm complete integration of the gene constructs and to detect transgene specific siRNA's are in progress.

GENETIC TRANSFORMATION OF GRAPEVINES WITH *Trichoderma harzianum* AND PEPTIDE GENES FOR THE IMPROVEMENT OF THEIR FUNGAL TOLERANCE

P. Hinrichsen, M. A. Reyes, A. Castro, E. Blanchard, S. Araya, M. Garnier, *F. Reyes, *P. Dell'Orto, *M. Moynihan, H. Prieto and C. Muñoz

Lab. Biotecnología, Experim. Centre La Platina, INIA. Casilla 439-3; and (*) Fundación Chile. Santiago, Chile. phinrich@platina.inia.cl

One of the main problems for grape growers is the atack by a very diverse mycological flora, among which the grey mold caused by *Botrytis cinerea* is the most severe and difficult to control in Chile. Botrytis causes losses in pre- and post-harvest, and even low levels of infection can have severe impacts on the value of table grapes. We initiated a genetic transformation approach to improvement of resistance to fungal diseases three years ago, based on a combination of *Trichoderma harzianum* anti-fungal genes and anti-microbial peptide genes introduced into selected table grape cultivars including Thompson seedless. Transformation of embryogenic cultures was initially optimized using GFP, and then combinations of antifungal genes were introduced using *Agrobacterium*-mediated transformation. More than 140 PCR-tested lines are under evaluation in a containment greenhouse. Preliminary evaluations based on inoculation of leaves with Botrytis indicate a significant level of resistance for some of the transgenic lines. After additional characterization of the transgenic lines we plan to evaluate the resistance of selected lines in field trials under isolation conditions.

Financed by Grants FONDEF-Chile # D99I-1001 & # D01I-1064.

TRANSGENIC GRAPEVINE PLANTS EXPRESSING GREEN FLUORESCENT PROTEINS TARGETED TO THE APOPLAST AND THE VACULAR SODIUM ANTIPORTER ATNHX1

Cecilia Aguero, Abhaya Dandekar, Eduardo Blumwald, and Carole Meredith

Departments of Pomology and Viticulture and Enology, University of California, Davis, CA 95616-8749, USA Fax 530-752-8502, E-mail: cbaguero@ucdavis.edu

Pierce's disease (PD), caused by the bacterial pathogen *Xylella fastidiosa*, is one of the most destructive diseases of grapevine. Because *X. fastidiosa* is xylem limited, any anti-*Xylella* gene product must be secreted into the xylem in an effective concentration to be useful as a therapeutic. In order to study protein secretion in grapes, pre-embryogenic calli originating from anthers of *Vitis vinifera* L. cvs. 'Thompson Seedless' and 'Chardonnay' were transformed via *Agrobacterium tumefaciens* with three gene constructs that include a gene that codes for a synthetic green fluorescence protein (GFP) and two types of fusion of GFP with the amino-terminals of the secreted protein trichosanthin (TCS) and the xylem specific protein XSP-30, all under the control of the CaMV 35S promoter. A strong fluorescence was found in embryos, roots, stems and leaves of plants transformed with GFP and XSP-30-GFP but the levels of fluorescence observed in TCS-GFP transformants were very low. In all cases fluorescence was detected only inside the cells. Western blotting analysis of apoplastic fluid is underway. In addition, we will show results obtained with the transformation of 'Thompson Seedless', 'Chardonnay' and rootstock '110R' with the vacular sodium antiporter gene *At*NHX1, which has been shown to improve salinity tolerance in transgenic tomato.

GENETICALLY CUSTOMIZED SEEDLESS GRAPES FOR FRESH MARKET INDUSTRY

Violeta Colova-Tsolova[a], Jiang Lu[a], and Avihai Perl[b]

[a]*Center for Viticulture & Small Fruit Research, Florida A& M University, 6505 Mahan Drive, Tallahassee, FL 32317, USA*
[b]*Department of Fruit Tree Sciences, Agricultural Research Organization, The Volcani Center, P.O.Box 6, Bet Dagan 50250, Israel*

The common muscadine grape, *Vitis (Muscadinia) rotundifolia* Michx., is native to the southeastern United States and has been cultivated for more than 400 years. Although the production of this species is currently relatively small, there is a tremendous opportunity for commercial growth based on increasing consumer interest in healthier diets and changing lifestyle. The discovery of high levels of anti-oxidant compounds in muscadine juices and wines has brought more attention to the muscadine grape, not only as an alternative cash value crop for the Southeast, but also as a new health food. Seedless muscadine grapes are likely to become another very important booster for the regional small fruit industry. Due to genetic incongruity and hybrid infertility between muscadine grapes and other varieties, not much progress has been made with the introgression of the seedlessness trait from *vinifera* grapes into the *muscadine* grape line using sexual hybridization. In light of these limitations, it is an efficient strategy to transfer individual traits as single genes into an already available and desirable genetic background, with a minimum disturbance to the original genome using DNA recombinant technology. The implementation and successful commercialization of genetically improved grape varieties will only be realized if number of obstacles, both scientific and otherwise, can be overcome. Additionally, avoidance of the use of antibiotic resistance genes as selective markers may become crucial for the possible release of the genetically modified crop. The presented study is designed to produce a new generation of genetically tailored seedless muscadine grapes that will provide biosafety guarantees and meet certain legal criteria for field release and commercialization. Gene conferring seedlessness has been transferred into the muscadine variety "Fry" and 17 independent transgenic clones are under greenhouse evaluation. The most important outcomes of this research are: for the first time, genetic transformation of muscadine grape has been accomplished; the feasibility of a gene transfer approach for genetic improvement of muscadine grape has been proven.

ENGINEERING DURABLE VIRUS RESISTANCE IN GRAPEVINE: GENERATION AND EXPRESSION OF SPECIFIC RECOMBINANT ANTIBODIES (SCFV)

Pascal Cobanov[a], Greta Nölke[b], Martin Orecchia[b], Pasquale Saldarelli[c], Mariangela Dell´Orco[c], Angelantonio Minafra[c], Giovanni Martelli[c], Rainer Fischer[b,d], Stefan Schillberg[d], Götz M. Reustle[a]

[a]*Center for Green Gene-Technology, Breitenweg 71, 67435 Neustadt, Germany. Contact: pascal.cobanov@dlr.rlp.de*
[b]*RWTH Aachen, Institut for Biology VII, Worringerweg 1, 52074 Aachen, Germany*
[c]*Plant Virology Laboratory, Departement of Plant Protection, Via G. Amendola 165/A, 70126 Bari, Italy*
[d]*Fraunhofer-Institut for Molecular Biology and Applied Ecology (IME), 52074 Aachen, Germany*

Grapevine (*Vitis* spp.) represents one of the major horticultural crops grown in temperate climates. Unfortunately many diseases can affect grapevine. Insects and nematodes are viticultural pests causing several damages on rootstocks and *Vitis vinifera* cultivars. Grapevines are susceptible to many viral diseases. Mealybugs are responsible for the transmission of closteroviruses and ampeloviruses, while nematodes transmit nepoviruses though the roots. ArMV and GFLV are two nepoviruses widespread in German vineyards and they are responsible for important economic losses. The viral infections decrease particularly both yield and quality of grapes. Unfortunately no chemicals are available to protect against viral infections and there is no natural resistance in grape against viruses. Therefore we are trying to establish durable virus resistance in grape though biotechnology. Antibody-based resistance in grape was chosen for engineering durable virus resistance in grapevine. Pathogen-specific recombinant antibodies expressed in plant cells of several species have been proven to be a good approach to affect pathogen infectivity and to engineer pathogen resistance. The aim of our project is the production of transgenic grapevines expressing recombinant antibodies (scFv´s) able to induce viral resistance in grapevine. Different types of antigens were chosen: virus particles, coat protein, movement protein or replicase, the first enzyme expressed for the replication of the virus. Recombinant antibody binding to conserved functional domains of viral proteins, such as replicase, lead to broad spectum resistance to viral pathogens by inactivating the targets inside the cell though immunomodulation. In this report we show the generation and characterisation of virus-specific recombinant antibodies and the Agrobacterium-mediated transformation of grapevine to produce transgenic plants resistant to major grapevine viruses.

MOLECULAR CHARACTERIZATION OF TRANSGENIC GRAPEVINE PLANTS

I. Gribaudo[a], G. Gambino[b], and M. Laimer[c]

[a]*Istituto Virologia Vegetale CNR, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
[b]*Dipartimento Colture Arboree, Università di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
[c]*Plant Biotechnology Unit, Institute of Applied Microbiology, BOKU, Nussdorfer Lände 11, Wien, Austria*

In the frame of a project aimed to obtain grapevine plants resistant to the Grapevine FanLeaf Virus (GFLV), the GFLV coat protein gene was inserted in three *Vitis vinifera* cultivars. Embryogenic calli were obtained from immature anthers and ovaries of the cultivars Nebbiolo, Lumassina and Blaufränkisch. Two binary vectors were used for transformation, carrying the full-length GFLV CP gene with an introduced start codon (pGA-CP+), or the same gene construct in antisense orientation (pGA-AS). The protocol adopted for selection and regeneration of transgenic embryos relied on prolonged culture on kanamycin (100 mg/l)-containing media. Forty-three lines of putatively transformed grapes were obtained, each deriving from single somatic embryos: 40 lines of Nebbiolo/pGA-CP+, one line of Lumassina/pGA-CP+, and two lines of Blaufränkisch/pGA-AS. The transgenic status of all lines was confirmed by Southern blot analysis. The number of T-DNA copies inserted in the genome ranges from 1 to 3. Digestion of genomic DNA with two different restriction enzymes (*Hind*III and *Eco*RI) showed that several lines share same hybridization patterns: the 40 lines of Nebbiolo likely derive from 8 independent transformation events. Results from Southern blot and PCR analyses indicated that a group of those lines (from one transformation event) probably contains a single incomplete copy of T-DNA, in which part of the CP gene (3` region) was deleted. No evidence of methylation of the transgenes at cytosine residues was found by Southern analysis of DNA digested with methylation-sensitive restriction enzymes. RT-PCR and Northern analysis showed the presence of the specific mRNA in all lines except for those that do not contain an intact T-DNA copy. Expression of GFLV CP gene was detected by DAS-ELISA: some lines accumulated the coat protein at low level while in others the protein was not detectable by ELISA test.

DISEASE RESISTANCE ANALYSES OF TRANSGENIC GRAPEVINES THAT CONTAIN ENDOCHITINASE OR ANTIMICROBIAL PEPTIDE GENES

J.R. Kikkert, J.R. Vidal, P.G. Wallace, S. Garcia-Zitter, W.F. Wilcox, D.M. Gadoury, R.C. Seem, T.J. Burr and B.I. Reisch.

Departments of Horticultural Sciences and Plant Pathology, New York State Agricultural Experiment Station, Cornell University, 633 W. North Street, Geneva, NY 14456-0462 USA

Most cultivars of *Vitis vinifera* L. are highly susceptible to several major grapevine diseases. We sought to develop disease-resistant forms of important cultivars by genetic engineering with an endochitinase gene (*ThEn42*) from *Trichoderma harzianum*; and the following antimicrobial peptides: a natural magainin gene (*MagII* from *Xenopus laevis*), a synthetic magainin derivative (*MSI-99*), and a gene from the peptidyl-glycine-leucine class (*PGL* from *X. laevis*). Eighteen endochitinase-expressing lines and three negative control lines of 'Merlot' were sprayed with a spore suspension of *Uncinula necator* (grapevine powdery mildew) in the greenhouse. The number of *U. necator* colonies that developed 12 days after inoculation varied among lines, but was not well correlated with chitinase expression. Notably, production of cleistothecia was significantly reduced in endochitinase-transgenics compared to non-transgenics. Six of 25 'Chardonnay' lines were slightly more tolerant to powdery mildew after natural infection in the field, but none of 41 'Merlot' lines showed significant resistance. Flowers at late bloom were sprayed with a spore suspension of *Botrytis cinerea* (bunch rot). During two years of field-testing, 3 of 4 high expressing endochitinase lines of 'Chardonnay' had reduced *Botrytis* symptoms. Similarly, after 1 year of field testing, 3 of 5 'Merlot' lines exhibited resistance to *Botrytis*. Thirty-six 'Chardonnay' lines that were positive for magainin type and/or PGL genes by PCR and dot blot analyses were spray-inoculated with *U. necator* spores in the greenhouse. Five lines showed moderate reduction of powdery mildew symptoms. Thirty-one of the magainin- and/or PGL-containing 'Chardonnay' lines were wound inoculated with *Agrobacterium vitis* (crown gall) in two separate experiments. Twenty-one lines had significantly reduced gall size. Antimicrobial peptide gene transcription was positively correlated with crown gall resistance. These vines are undergoing further tests for powdery mildew, Botrytis bunch rot, crown gall, and black rot resistance in the greenhouse and field.

EVALUATION OF EXOGENOUS DNA BY QUANTITATIVE REAL-TIME PCR IN TRANSGENIC GRAPE

Federica Savazzini, Lorenza Dalla Costa and Lucia Martinelli.

Istituto Agrario San Michele all'Adige, 38010 San Michele all'Adige (TN), Italy, Lucia.Martinelli@ismaa.it

Quantitative real-time PCR has proven to be a powerful tool to accurately estimate transgene copy number and exogenous gene expression in plants. Compared to Southern and Northern blot analysis, accuracy, rapidity and low-cost are the main advantages of real-time PCR. However, this technique requires important preliminary work for standardizing and optimizing the many parameters involved in the process. Unfortunately, grapevine data on this topic are not presently available in the literature. We have developed a method for properly performing quantitative analysis in grapevines where the genes for NPTII, ß-glucuronidase, GVA antisense movement protein, and phosphomannose isomerase where transferred. First, we identified the optimal endogenous genes to be applied as referee on grape, for DNA quantification and characterization and for gene copy number detection. In order to estimate the transgene copy numbers, we used an approach based on a grape transgenic line at a known insertion number with a method based on synthetic hybrid amplicons. The amplicons may contain both transgenes and reference endogenous gene target sequences. For optical detection, TaqMan and SYBER Green 1 probes were exploited with the aim of comparing two different florescent reporters characterized for having specific and non specific nature, respectively. Validation of our method was verified with Southern blot analysis comparison. A similar study is also in progress for RNA expression quantification. The results of our analysis will be discussed in the paper.

CLONAL POLYMORPHISM IN THE RED WINE CULTIVARS CARMENERE AND CABERNET SAUVIGNON

X. Moncada, L. Muñoz, M.H. Castro, *D. Merdinoglu and P. Hinrichsen

Lab. Biotecnología, Experimental Centre La Platina, INIA. Casilla 439-3, Santiago, Chile and (*) INRA-Colmar, France. phinrich@platina.inia.cl

The cultivar Carmenère originated in the Médoc region of France. It is included in the "Carmenet" family together with Cabernet Sauvignon, Cabernet franc, Merlot and others. Its presence in French vineyards was dramatically reduced due to the Phylloxeria crisis starting the middle of the 19th Century. During the last decade, no more than 10 ha of this cultivar was found in France. In contrast, Carmenère was re-discovered in 1994 in Chile, where it is cultivated and has been confused with Merlot. Presently, there are more than 5,000 ha of this cultivar in the Central Valley of Chile. In the present study we analyzed the clonal polymorphism of Carmenère using microsatellites and AFLP markers and compared the results with Cabernet Sauvignon. We analyzed 56 Carmenère clones from clonal selection assays conducted in Chile as well as nine clones from France and one from Italy. Using 18 microsatellite markers we found two polymorphic *loci*. VVMD7 grouped 66% of the clones with a specific allelic combination and 33% with another, while VMC5g7 showed a mutation for one allele in one clone. AFLP analysis carried out with the Chilean clones using 13 primer combinations indicated 2.3% polymorphism. Some of these amplicons are under cloning and sequencing. In contrast, the analysis of 58 clones of Cabernet Sauvignon with 91 microsatellite markers showed 16 polymorphic *loci*. The analysis of 46 of these clones with AFLP found 5.9% clonal polymorphism. These results indicate that Carmenère could present a low level of genetic diversity, an important factor to be considered in future of clonal selection.

Financed by Grants FONDEF-Chile # D00I-1138 and Conicyt/France Embassy Fellowship Program

SEGREGATION OF TENDRIL DISTRIBUTION PATTERNING IN GRAPEVINE POPULATIONS

Peter Cousins, Jason Coburn, and Jennifer Vidmar

USDA-ARS, Plant Genetic Resources Unit, Cornell University, Geneva, New York, United States of America

Variation in phyllotactic patterning of tendril and cluster distribution is recognized as a varietal characteristic in grapevine. We examined the segregation of tendril distribution patterns in seedling populations derived from self-pollination of interspecific *Vitis* hybrid varieties. Seedlings of populations resulting from Buffalo, Caco, Clinton, Golden Muscat, and Ives controlled self-pollinations were grown in a greenhouse and evaluated for tendril patterning at about twelve weeks of age. We scored the presence or absence of tendrils or equivalent accessory organs beginning at the first node bearing a tendril and continuing for a total of nine successive nodes. We assigned a numerical score to each seedling equivalent to the number of tendrils present in nine nodes. Populations differed significantly in their tendril distribution patterns. Variation in tendril patterning was highest in the population of self-pollinated Clinton seedlings. The segregation of tendril patterning indicates the action of one or a few major genes conditioning tendril distribution patterning in grapevine. Since tendrils and clusters are developmentally related, genes influencing the distribution patterning of tendrils may also influence the number and distribution of clusters.

STEPS TOWARD UNDERSTANDING THE MOLECULAR BASIS OF SEEDLESSNESS IN STENOSPERMOCARPIC GRAPES

Uri Hanania, Margarita Velcheva, Nachman Sahar, and Avihai Perl

Department of Fruit Tree Sciences, Agricultural Research Organization, The Volcani Center, P.O.Box 6, Bet Dagan 50250, Israel

Seedless forms of *Vitis vinifera* grapes have been highly prized as fresh and dried fruits for centuries. Grape breeders are making enormous efforts in developing new seedless cultivars. During the last years we produced transgenic seedless grapes by expressing 2 different "suicide" genes under the control of different seed specific promoters in two different seeded cultivars. These procedures will not substitute ongoing breeding efforts but can provide additional powerful means to understand the molecular basis for seedlessness in grapes. Seedless berries develop either through parthenocarpy or through ovule abortion at an early stage of development. The latter type is termed stenospermocarpy. Stenospermocarpic cultivars comprise 85% of the fresh table and raisin grape market. For these cultivars pollination and fertilization are both required to obtain fruit set. The berries contain aborted seeds (seed traces) of various sizes. Although estimates vary as to the number of abnormal ovules or embryo sacs, at least some are morphologically functional enough to accomplish fertilization. Natural mutant shoots on seeded vines of different cultivars were the first available breeding material for producing new seedless cultivars. We have utilized such two lines, the commercial seedless Thompson and a seeded Thompson. Genomic studies followed by substraction libraries compared these two lines from budbreak till 30 days prior to anthesis. Several transcriptional factors and chaperonins involved in embryo formation and abortion were found to be differentially expressed in either the seeded or seedless lines. The presentation will describe preliminary results obtained with transgenic grape and tomato plants expressing RNAi of one of these chaperonins under both constitutive and inducible promoters.

INTEGRATING MOLECULAR AND PHYSICAL MAPS IN GRAPEVINE (*VITIS VINIFERA* CV. PINOT NOIR): POWERFUL AIDS FOR GENE ISOLATION AND BREEDING

R. Velasco[a], M.S. Grando[a], M. Troggio[a], G. Faes[a,d], M. Pindo[a], G. Malacarne[a], C. Segala[a], G. Coppola[a], P. Fontana[a], J. Zambanini[a], T. Jesse[b], B. Chaloub[c], A.F. Adam-Blondon[c], G. Prete[d], S. Scalabrina[d], N. Felice[d], R. Marconia[d], M. Moroldo[a,d], M. Morgante[d]

[a]*Istituto Agrario San Michele all'Adige, via Mach 1, 38010, San Michele, Trento, Italy*
[b]*Keygene N.V. PO Box 216, 6700 AE Wageningen, The Netherlands*
[c]*INRA-URGV, 2 rue Gaston Crémieux, CP 5708, 91057 Evry, France*
[d]*Dipt. Scienze Agrarie ed Ambientali, Universita' di Udine, via delle Scienze 208, 33100 Udine, Italy*

Grapevine breeding will benefit enormously from new molecular tools being developed once the grape genome has been completely sequenced. The past 5 years have seen a strong effort by a number of research groups to produce several molecular maps that are currently on the way of integration using common markers (mainly microsatellites). The next steps are focused on physical mapping and the integration of the two approaches. Together, they will pave the way to the complete sequencing of the genome. Even in the absence of a complete genome sequence, physical mapping offers new opportunities for the map-based cloning of genes following QTL analysis or segregation analysis of monogenic traits. In the present work we will present the progress on (i) the construction of a large BAC library of Pinot noir (clone ENTAV 115; 66.500 clones, 110 kb average size, 14x equivalents), (ii) the development of a reference molecular map from the cross *V. vinifera* cv. Syrah x *V. vinifera* cv. Pinot noir, (iii) the positioning of several hundred molecular markers on the BACs, (iv) the production of several thousand BAC fingerprints and the assembly of a contig map and (v) the production of BAC end sequences. All these activities have been carried out in order to produce an integrated genetic and physical map that will be available to the public .

GRAPEVINE POWDERY MILDEW: MODULATION OF HOST GENE EXPRESSION BY A BIOTROPHIC PATHOGEN

Matthew A. Hayes and Ian B. Dry

CSIRO Plant Industry, Horticulture Unit, PO Box 350, Glen Osmond, SA 5064, Australia; University of Adelaide, School of Horticulture & Wine, Glen Osmond, SA 5064 and Cooperative Research Centre for Viticulture, PO Box 145, Glen Osmond, SA 5064, Australia

The powdery mildews are biotrophic pathogens that derive all nutrition from a specialised feeding structure, the haustorium, that forms within epidermal cells of the host. The causative agent of grapevine powdery mildew, *Uncinula necator*, has previously been reported to increase the glucose content of host tissue in the locale of the infection lesion, suggesting the pathogen exerts some influence on host metabolism. To investigate aspects of sugar metabolism and movement during powdery mildew disease of grapevine, a real-time reverse transcription PCR approach was used to study the expression of invertases and sugar transporters in RNA isolated from infected and non infected grapevine tissues. These studies indicate that a cell wall invertase, one of four hexose transporters and a putative ferrous iron transporter are strongly upregulated in powdery mildew infected tissues compared to control (non-infected) tissues. The expression of these genes was also found to be upregulated in leaves infected with the grapevine downy mildew fungus *Plasmopara viticola*. To investigate the specificity of the observed gene induction, the expression of these genes was analysed in distinct powdery mildew lesions and measured relative to non-infected regions of the same leaf. In this analysis, the cell wall invertase, hexose transporter and iron transporter were not significantly induced. This observation indicates that the upregulation is a non specific response to infection that may not play a direct role in nutrient delivery from host to fungus at the biotrophic interface, but may alter the usual source – sink status of the whole leaf. To investigate possible signalling mechanisms underlying these responses, leaves were cultured with abscisic acid, salicylic acid and auxin, RNA extracted and analysed as before. Culture with auxin for six hours induced the cell wall invertase and hexose transporter induced by powdery and downy mildew infection, but also upregulated another hexose transporter and down regulated GIN1, a vacuolar invertase. This study provides evidence that powdery mildew infection of grapevine modulates the expression of genes involved in sugar metabolism and transport, presumably creating a metabolic environment better suited to nutrition of the pathogen. The mechanisms and signalling events underlying these observations are yet to be determined.

PORTUGAL AS A CENTRE OF AUTOCHTHONOUS *VITIS VINIFERA* GENES

H. Joerg Boehm

Viveiros Plansel S.A., Quinta de Sao Jorge, Apartado 2, Portugal

The Portuguese Grape industry has advanced after integration into the European Union. Selection work on indigenous varieties was conducted and 341 different cultivars were classified. From this pool 90% were autochthonous. By natural selection, the Portuguese grapevine gene pool became highly adapted to a hot climate similar to other southern European countries. Significant distinction to Spanish and French varieties has been found by molecular characterisation. Due to the lack of grapevine selection in Portugal, full intra-varietal variability still exists in all varieties. Selection work began in early 1980s for 30 varieties on a large scale (250 cultivars by each variety). A program for the characterisation of distinct clones of the most important varieties by molecular techniques is proceeding. Genetic evaluation for productivity was conducted at different locations for a period of time, resulting first in a polyclonal selection program and later in clonal selection and certification. Certification work in Portugal has been established both by the governmental services directed mainly to the conservation of large scale biodiversity and by private organizations focusing basically on the guaranty of clean stock, according to ICVG rules. Diagnosis has been based on Indexing and ELISA accompanied by PCR techniques with specific markers for Nepo, Fovea and Viti viruses, a total of 17 viruses. Presently, there are more than 120 clones of 25 varieties admitted to the CE certification-scheme. Initial material is produced from foundation blocks at isolated locations. Multiplication of the selections has been performed in greenhouses, by the private sector *in situ* with three consecutive stages subdivided into sanitary families. There exists certified material of all selected varieties. Within Portugal, the varieties are classified according to different wine growing regions based on the quality of wines made or tradition. Work is proceeding on disease resistance by breeding techniques and on the identification of resistance genes, by molecular analysis. Mono-varietal wines and regional blends in last decade began to win prizes at international wine competitions. More and more the "new world" wine regions are interested in varieties coming from Portugal, adapted to hot climates.

ROOT SURVIVORSHIP UNDER DEFICIT AND DRYLAND FARMING CONDITIONS FOR 1103P AND 101-14 MGT ROOTSTOCKS IN THE OAKVILLE REGION OF THE NAPA VALLEY

David R. Smart, Taryn L. Bauerle, Christine Stockert and David M. Eissenstat

**Department of Viticulture & Enology, University of California, One Shields Avenue, Davis, CA 95616, USA [Phone: 530-754-7143; Fax: 530-752-0382; Email: drsmart@ucdavis.edu]*

The most commonly accepted phenology for root growth by *Vitis* is bimodal, with one peak of root growth at flowering and another peak following harvest. We are examining the influence of both deficit irrigation and dryland farming, and timing of fertilizer application on two rootstocks that differ in overall vigor (1103P and 101-14 Mgt). The scion is Merlot and the vineyard is located at Oakville in the Napa Valley on Bale (variant) gravelly loam (Fine-loamy, mixed, superactive, thermic Cumulic Ultic Haploxeroll). The deficit irrigation treatments are as practiced in this region, using a deficit threshold at stem water potentials of approximately –1.0 MPa. The project has been continuing for two years. Root population dynamics were continually monitored on seasonal cohorts of roots using a digital imaging camera inserted into clear minirhizotron tubes to a depth of approximately 1.4 meters. One minirhizotron tube was placed within the drip wet-up zone and another on the opposite side of the vine where no water was received. For the dryland treatments, two minirhizotrons were positioned in at the same spatial coordinates on either side of the vine. New roots emerging during the Spring root flush were produced between 30 to 90 cm from the soil surface on the irrigated side of the vine. We observed significant differences between the performance of 1103P and 101-14 Mgt rootstocks in response to irrigation deprivation. Under dryland conditions, 1103P rootstock had greater root survivorship than 101-14 Mgt. Root survivorship also declined under deficit irrigation for both rootstocks. There was a strong interaction among irrigation deprivation and fertilization treatments when water and potassium nitrate were applied following harvest. In other words, deficit irrigated vines produced more roots when irrigated following flowering or after harvest, but only if fertilized. Interestingly, in this soil and the Mediterranean climate conditions of the region we did not observe a substantial Fall root flush. Some new roots were produced, but very few in comparison to the strong Spring root flush we have observed.

RESPIRATION ACTIVITY IN DIFFERENT ABOVE-GROUND ORGANS OF *VITIS VINIFERA* L. ACCORDING TO TEMPERATURE AND DEVELOPMENTAL STAGE

A. Palliotti[a], A. Cartechini[a], O. Silvestroni[b] and S. Mattioli[b]

[a]*Dept. of Arboriculture and Plant Protection, University of Perugia, Italy*
[b]*Dept. of Environmental and Crop Science, Marche Polythecnic University, Italy*

Respiration activity, which may utilize up to 70% of daily CO_2 fixed via photosynthesis, is poorly defined in grapevine. It is well known that the specific respiration rates per weight or surface units changed during the season according to organs, substrate availability, stage of growth and environmental factors. Temperature is one of the most important factors influencing respiration activity during the season. The purpose of this study was to characterize the change respiration rate to temperature during the season for different above-ground organs in grapevine. Two key goals were to calculate of different stages of development (i.e. 3 weeks after bud burst, flowering, veraison and ripening) the coefficients "a" and "k" describing the logarithmic response of respiration rate to temperature ($R = a \cdot e^{(k\,T)}$), in leaves, stem, cluster and old wood (2-3 year-old spur) and to then apply these coefficients to mature vines at the same developmental stage. The change of respiration rate as a function of temperature was measured on 4-year-old Sangiovese/Kober 5BB grapevines, grown outside in 40 l pots, and subjected to incremental increases in temperature in a 12 m^3 climate controlled chamber. A 5 °C heating steps from 10° to 35 °C were imposed in completely dark condition. Respiration was measured using an open system LCA-3 portable infrared gas analyser (ADC, UK) and appropriate assimilation chambers. The respiration coefficients were then applied to mature vines of cv. Sangiovese/Kober 5BB sited in central Italy (Perugia, lat. 42°58'N, long. 12°40'E) trained to spur-pruned cordon system and winter pruned retained about 16 buds per vine. Daily, total respiration data were obtained by multiplying the respiration rates to surface area in leaf, stem and old wood and to fresh weight in the cluster. At each developmental stage, the daily total respiration, expressed as mg CO_2 $vine^{-1}$ day^{-1}, was computed as the sum of leaf, stem, cluster and old wood respiration rates. Changes of respiration rate according to temperature and developmental stage in leaf, stem, cluster and old wood of open field grapevine will be reported and discussed.

EFFECT OF DROUGHT ON PARTITIONING OF ^{14}C-LABELLED PHOTOSYNTHATE IN *VITIS VINIFERA* L.

Josefina Bota[a*], Jaume Flexas[a], Hipólito Medrano[a†] and Oleg Stasyk[*b]

[a]*Departamet de Biologia, Universitat de les Illes Balears, Carretera de Valldemossa Km 7,5,CP 07122, Palma de Mallorca. Spain*
[b]*Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, Vasylkivska Str. 31/17, 03022-Kyiv-22-Ukraine*

Water stress effects on export and distribution of photosynthates were studied in grapevine using ^{14}C-labeling. Plants of *Vitis vinifera* L. cv. Tempranillo, with and without fruit, were subjected to moderate and severe drought. Additionally, the cv. Alfonso Lavallée with fruit were subjected to severe drought. Drought treatments resulted in a significant reduction of predawn leaf water potential, net photosynthesis and stomatal conductance. The presence of fruit in Tempranillo plants strongly stimulated carbohydrate export in all treatments. The imposition of moderate or severe drought treatment in Tempranillo plants resulted in a decreasing, although non-significant carbohydrate export out of the fed leaves, and did not affect the distribution of ^{14}C-photosyntates to different plant parts and the ^{14}C-distribution into different fractions of fruit components. In contrast, drought responses of Alfonso Lavallée vines resulted in a highly significant reduction of export and the photosynthate distribution pattern was also altered. The present results suggest than in Tempranillo water stress caused important reductions on source activity (photosynthesis), but sink activity was less altered. In contrast, water stress of Alfonso Lavallée vines altered the partitioning pattern primarily through reductions in the strength of fruit.

FROST HARDINESS OF IRRIGATED AND FERTIGATED CHARDONNAY VINES

Borbála Bálo[1], Sándor Misik[1], Erzsébet Miklós[2], Ildikó Király[1] and Gyula Váradi[2]

[1]*Research Institute for Viticulture and Enology, 3301 Eger, P.O.B. 83, Hungary.*
[2]*Research Institute for Viticulture and Enology, 6001 Kecskemét, P.O.B. 25. Hungary.*

The purpose of this study was to develop irrigation and/or fertigation practices to improve grape production, fruit and wine quality and vine cold hardiness in the 'Eger wine region' of Hungary. Cordon trained Chardonnay vines with two different pruning levels were irrigated or fertigated in 2002 and 2003. Irrigation and fertigation were carried out from bloom until 2 weeks prior to harvest with Netafim pressure compensated in-line drippers. Changes in soil matric potential were followed by tensiometers. Soil and leaf nutrient status was measured at bloom, veraison and harvest. Different methods were used to characterize the dormancy period of buds and canes: (1) microwave technique measuring free and bound water content of canes, (2) freezing chamber test evaluating the bud's survival at a given temperature (e.g. -17°C or -19°C) and (3) differential thermal analysis exploring the median low temperature exotherm (MLTE) values. Yield of irrigated or fertigated vines exceeded that of the control by 30-50 and 10-30% in 2002 and 2003, respectively, depending on pruning level. Both irrigation and fertigation slightly retarded (by about 1 month) cane ripening, which was reflected in the ratio of bound to total water content of the canes. Control vine buds manifested higher frost tolerance in November and December but the advantage of irrigation and fertigation became evident by the end of deep dormancy. No significant difference occurred in bud frost hardiness due to different pruning levels under the conditions of this study.

ENDOPHYTIC COLONIZATION OF *VITIS VINIFERA* L. BY A PGPR, *PSEUDOMONAS* SP. STRAIN PSJN THAT PROTECTS GRAPEVINE AGAINST *BOTRYTIS CINEREA*

S. Compant[a], E. Ait Barka[a], B. Reiter[b], A. Sessitsch[b], J. Nowak[c] and C. Clément[a]

[a]*Laboratoire de Stress, Défenses et Reproduction des Plantes, URVVC UPRES EA 2069, Université de Reims Champagne-Ardenne, UFR Sciences, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France*
[b]*Department of Biotechnology, Division of Life and Environmental Sciences, ARC Seibersdorf research GmbH, A-2444 Seibersdorf, Austria*
[c]*Department of Horticulture, Virginia Polytechnic Institute and State University, 0327-301 Saunders Hall, Blacksburg, VA 24060, USA*

Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria having a beneficial effect on plants. They can enhance emergence, stimulate growth, act as biocontrol agents and live as endophytes. In a previous study, a non-fluorescent and non-siderophic PGPR, *Pseudomonas* sp. strain PsJN, was shown to promote grapevine growth and an effective biocontrol agent against *Botrytis cinerea*. Nevertheless, little is known about the distribution of PsJN in grapevine. Such information may help to clarify the means by which *Pseudomonas* PsJN protects plants against fungal diseases. The dynamics of *Pseudomonas sp.* strain PsJN were studied in *Vitis vinifera* L. cv. 'Chardonnay' plantlets using *gfp* and *gus*-marked bacteria. The rhizosphere of five weeks-old plantlets with five leaves was inoculated under gnotobiotic conditions. Then, dilution plating assays and optical microscopy with *gfp* and *gus*-marked bacteria were used to evaluate the colonization pattern. Both strains were detected on rhizoplane, in root internal tissues, in the fifth internode and the fifth leaf tissues reaching respectively 9.1, 6.9, 5.8 and 6.6 log cfu.g-1of fresh weight, 96 h after inoculation with bacterial populations. Visualization of *Pseudomonas sp.* strain PsJN demonstrated that bacteria are localized on rhizoplane, close to the cell walls of rhizodermis, at root emergence sites and in all root internal tissues. Furthermore, bacteria were not observed at stem and leaves surfaces but were found in xylem vessels of the fifth internode and the fifth leaf of plantlets as well as in stomata. Our results demonstrated endophytic colonization of *Vitis vinifera* L. by *Pseudomonas sp.* strain PsJN. It appears that bacteria move upwards after rhizosphere inoculation and colonize stem and leaves internal tissues of plantlets. Moreover, bacteria population seems to be more important in leaves than in stem, suggesting that this PGPR colonizes the phyllosphere allowing the use of sub-stomatal chambers for multiplicity.

IN VITRO GRAPEVINE AS A MODEL FOR MONITORING THE EFFECT OF EXCESSIVE LIGHT BY REAL-TIME PCR

L.C. Carvalho[a], P. Vidigal[a], B.J. Vilela[a], S. Amâncio[a] and P. Mullineaux[b]

[a]*DBEB/CBAA, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal*
[b]*DDSB, John Innes Centre, Colney, Norwich NR4 7UH, UK*

Many environmental stresses, such as excessive light, can exert at least part of their effect through oxidative damage. Consequently, the antioxidant defense system of plants is attracting considerable research interest. A model for studying physiological responses to excessive light is that obtained by transferring *in vitro* plantlets to *ex vitro* conditions under irradiances several fold higher than the irradiance applied during *in vitro* growth. In this work we present the response to photo-oxidative stress of grapevine plantlets during the first seven days after transfer to ex vitro. For that purpose we have monitored 1) the total activities of anti-oxidative enzymes: catalase, glutathione S-transferase, superoxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase; 2) the expression of the transcripts for those enzymes by applying real-time PCR to *Vitis* cDNA with either the Arabidopsis or *Vitis* correspondent primers; 3) the levels of related metabolites: hydrogen peroxide, reduced/oxidized ascorbate, reduced/oxidized glutathione. We present evidence that excess light triggers the expression of anti-oxidative enzymes at the mRNA level, showing a peak 48 hours after transfer to *ex vitro*. Enzyme activities and metabolite levels, namely glutathione and ascorbate, were consistent with gene expression. After the peak at 48 hours, both mRNA levels and enzyme activities return to lower, steady values. From the results obtained with the present plant model system we will discuss its suitability for dissecting the molecular effect of photo-oxidative stress as well as its anti-oxidative efficiency.

INVESTIGATING THE POTENTIAL OF THERMAL IMAGING IN MONITORING STRESS IN GRAPEVINES

Olga M. Grant[a] and M. Manuela Chaves[b]

[a]*Instituto de Tecnologia Química e Biológica, Oeiras, Portugal*
[b]*Instituto Superior de Agronomia, Lisboa, Portugal*

Stomatal closure is considered a sensitive response to soil water deficits, and hence has potential as an indicator of plant stress and of value in irrigation scheduling. In particular, this would be of benefit where precise regulation of water supply is required, such as in the production of high-quality grapes for wine. In grapevines, reduced photosynthesis due to limited water availability is primarily a result of stomatal closure. However, the use of porometers to monitor stomatal conductance is time-consuming, labour-intensive, and gives only point measurements. As stomata close, energy dissipation is decreased and so leaf temperatures tend to rise. Therefore leaf or canopy temperature can be used as an indicator of plant stress. For some years, infrared thermometry has been used for irrigation scheduling, although the method is not that precise. The use of thermal imaging systems allows us to rapidly and non-invasively obtain more integrated data for individual leaves or areas of canopies. The nature of grapevine trellises, with plentiful leaves that are close to vertical exposure means that this crop may be particularly suited to monitoring with a thermal imager, which can be carried along the rows. While recent papers have emphasised the advantages of thermal imaging, experiments designed to test whether the technique is in fact as useful as more traditional physiological methods in distinguishing between stress treatments are lacking. We tested whether thermal imaging can be used to distinguish between irrigated and water-limited grapevines, both in the greenhouse and in the field. Where we concentrated on images of individual leaves, our results highlight a number of issues that could prevent thermal imaging from accurately distinguishing between stressed and non-stressed plants, particularly the effects of variation in leaf angle towards sunlight or the imager. However, in a field experiment, thermal images of sections of canopies successfully distinguished between non-irrigated and fully irrigated canopies, where porometer measurements failed to do so.

EFFECT OF IRRIGATION ON VEGETO-PRODUCTIVE BEHAVIOUR OF SAUVIGNON BLANC GRAPEVINE IN ITALY

P. Storchi, F. Giorgessi, A. Tarricone and F. Bonollo

Istituto Sperimentale per la Viticoltura, Conegliano (Italy), storchi@ispervit.it

The variation of different vegetative parameters and grape quality during the maturation period was studied in Sauvignon blanc in 3 different areas of cultivation (north, south and central Italy) with two different water regimes under irrigation and without irrigation. In 2003 soil water content, air temperature, irradiance, precipitation and ETP were monitored. Leaf water potential, photosynthesis, transpiration and stomatal conductance were measured once a week from bloom to harvest. Analysis of total acidity, pH, soluble solids and aroma potential was determined from veraison to harvest. Yield and cluster number were measured at harvest. Clear differences between areas and treatments were observed on vine physiology and must quality. Aroma analysis of grapes showed that moderate irrigation increased flavour components. Pyrazines content was influenced by climate, degree of grape ripeness and available water content of the soil. The results indicate the importance of irrigation for preserving grape quality of Sauvignon blanc in regions subjected to a soil water deficit combined with high temperature.

EFFECTS OF BUDBREAK TEMPERATURE ON SEASONAL SHOOT AND FRUIT GROWTH IN GRAPEVINES

Markus Keller[a], Lynn J. Mills[a], Julie M. Tarara[b] and John Ferguson[b]

[a]*Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350, USA*
[b]*USDA-ARS, Prosser, WA 99350, USA*

Bud temperature during budbreak of mature, field-grown Cabernet Sauvignon vines was modified using a forced-convection cooling/heating system. Four temperature regimes were applied individually to 10 exposed buds in 2002 and 2003: cool (ambient - 5°C), ambient, warm (ambient + 5°C), and hot (ambient + 10°C). Bud temperatures were monitored using thermocouples inserted in the bark around each bud. Treatments were applied from the beginning of sap flow until individual flowers were visible on inflorescences. Heating buds advanced budbreak and dramatically accelerated shoot growth, whereas cooling buds delayed budbreak and retarded shoot growth compared with ambient temperatures both seasons. Temperature-induced differences in vegetative development initiated during budbreak remained throughout the entire season and were due to greater vigor of the main shoot, stimulation of lateral growth, and increased leaf-area development. Heating buds also increased the proportion of lignified internodes by fruit harvest. In 2003, but not 2002, the hot treatment slightly reduced flower numbers per cluster, but the other treatments did not differ. Overall, variability in flower numbers was very high. Fruit set decreased with increasing flower number, but also increased with increasing bud temperature both seasons. Despite the decrease in berry numbers with lower bud temperature, bud cooling also reduced berry weights compared with the other treatments. Therefore, yields per shoot differed 2.3-fold between the cool and hot treatments. Despite the drastically reduced leaf area, the cool bud treatment resulted in the highest fruit sugar and color but lowest pH, whereas the warmest treatment led to the lowest sugar and color and highest pH across the two seasons. These results indicate that brief episodes of warm temperatures during budbreak dramatically stimulate seasonal vegetative growth, while effects on reproductive growth are minor and may be indirect due to differences in shoot growth.

A TALE OF TWO SEASONS – IMPACT OF VITICULTURAL PRACTICE ON RED GRAPE PHENOLICS

S-J. Bell, P. Sivilotti and P.B. Hoj

The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA, 5064, Australia

An investigation into the effect of vine vigour and and trellis system (Vertical Shoot Positioned-VSP and Scott Henry-SH) on the phenolic profile of Shiraz grapes was undertaken in the Barossa Valley, South Australia. The Barossa Valley is classified as a warm region. However, during this trial the climatic conditions were vastly different from the long term average (MJT=21.4C°). Grapes ripening in 2001 were subjected to very warm days and nights (MJT=28.2C°), whereas those in 2002 experienced much milder conditions (MJT=19.8C°). Determination of anthocyanins by HPLC analysis demonstrated that the effect of trellis system on berry composition was very dependent on the nature of the growing season. In the hot 2001 season, the more shaded VSP berries exhibited higher anthocyanin contents (mg/berry) than the more exposed SH berries. In the cool 2002 season, it was the more exposed SH berries that had higher anthocyanin contents than those from the more shaded VSP berries, during ripening. However, by harvest there was no difference between the anthocyanin contents of cool season VSP and SH berries. In both the hot 2001 and cool 2002 seasons, the concentration (mg/g berry weight) of anthocyanins from the more exposed SH berries exceeded that of the more shaded VSP berries. This was largely attributable to the lower weight of SH berries in comparison to VSP berry weight in each season. This research clearly illustrates that to optimize the benefits of particular trellis system, careful consideration of the long term climatic trends for that site is required. This, in combination with the ability to modulate the canopy characteristics via appropriate viticultural inputs, in response to the specific weather conditions in a given vintage is essential.

THE PHENOLIC COMPONENTS OF GRAPE BERRIES IN RELATION TO WINE COMPOSITION

Douglas O. Adams[a], James F. Harbertson[a], John C. Hazak[a], Ryan E. Hodgins[a] and Chin Ho Lin[b]

[a]*Department of Viticulture & Enology, University of California, Davis CA, USA*
[b]*Department of Botany, National Chung-Hsing University, Taichung, Taiwan.*

Most of the major solutes present in the grape berry at harvest contribute to wine composition in proportion to their amount in the fruit. However, many of the phenolic components are located in specialized tissues of the skin and seed, and because of differential extraction their representation in wine may not reflect their relative abundance in the fruit at harvest. By measuring the quantity of tannin in Cabernet Sauvignon fruit at two sites and comparing it with tannin concentration in the resulting wines, we found that 25 to 75% of the fruit tannin was extracted during winemaking. Further studies indicated that some of the non-extracted tannin was tightly bound to the insoluble matrix of the grape berry. We prepared a suspension of the insoluble cell fragments from Cabernet Sauvignon berries and determined its capacity to bind tannins at different times during ripening. Mesocarp material collected from fruit 40 days after veraison had more than five times the capacity for tannin binding compared to veraison fruit. The binding capacity appeared to decline as the fruit approached harvest. We also studied Cabernet Sauvignon at several commercial vineyards in Napa Valley and found that the amount of insoluble matrix in skins and mesocarp varied among different vineyards. We were able to determine that the capacity of the insoluble matrix to capture tannin can amount to more than a third of the tannin present in the fruit. This result indicates that tannin binding to the insoluble matrix of grape berries may be an important factor in the ability to extract tannin from fruit during fermentation.

COMPARISON AND OPTIMIZATION OF RNA EXTRACTION METHODS FOR GRAPE LEAVES

Elizabeth A.R. Tattersall[a], Ali Ergul[b], Fadi AlKayal, Grant R. Cramer[a]

[a] *University of Nevada, Biochemistry Dept., Reno, NV 89557*
[b] *Institute of Biotechnology, University of Ankara, 06500, Besevler-Ankara, TURKEY*

The high concentration of polyphenols and polysaccharides in grape leaves make it challenging to obtain high quality RNA from this organ. Many kinds of protocols have been developed for RNA extraction from difficult plant tissues. We have compared different methods of RNA extraction, including RNeasy™, tris-lithium chloride, hot borate, hot sodium acetate, guanidine thiocyanate, ultracentrifugation, Plant RNA Purification Reagent (Invitrogen), TRIzol™ and sodium perchlorate based methods. The addition of specific compounds to remove phenols and polysaccharides is critical for downstream applications such as PCR and gene expression studies. We assessed RNA quality using spectrophotometric methods and formaldehyde-agarose gel electrophoresis. RNA samples that passed these initial tests were used in RT-PCR reactions. There was a wide range of results depending upon the protocol used. Evaluations are based upon cost, ease of use, time to complete the extraction and quality of the RNA isolated. The tris-lithium chloride method is relatively time-consuming, but has given consistently high yields of good quality RNA, suitable for PCR and other applications. Invitrogen's Plant RNA Purification Reagent, when followed by a cleanup using Qiagen's RNeasy™ column also gave good yields, but in much less time. In contrast, TRIzol™ and guanidine thiocyanate methods did not yield usable RNA.

STUDY OF A NOVEL GRAPEVINE WRKY TRANSCRIPTION FACTOR AND ITS ROLE IN PLANT DEFENSE RESPONSES

R. Mzid[1]; C. Marchive[2]; L. Deluc[2]; V. Lauvergeat[2]; D. Blancard[3]; F. Barrieu[2]; S. Hamdi[2]; N. Drira[1].

[1]- *Laboratoire des Biotechnologies Végétales Appliquées à l'Amélioration Des Cultures, Faculté des Sciences de Sfax. Route de soukra, Km 3,5, BP 3018, Sfax, Tunisie.*
[2]- *Equipe Génomique Fonctionnelle et Qualité de la Baie de Raisin, UMR PBV 619 BP 81, 33 883 Villenave d'Ornon, Bordeaux, France.*
[3]- *INRA/UMR Santé Végétale, BP 81, 33 883 Villenave d'Ornon, Bordeaux, France.*

Biotic stress induce negative effects on both quantity and quality of grape production. In order to prevent the severe annual losses due to pathogen attack it is necessarily to closely investigate the natural plant defense strategies deploid against pathogens. In this report, we have focused on a plant specific transcription factors, called WRKY proteins, that is composed of more than 70 expressed genes in *A. thaliana*. It is involved in the regulation of several physiological processes such as pathogen defense, senescence, wounding and trichome development. They specifically bind to the W box motif (T)(T)TGAC(C/T) present in the promoter of PR (Pathogenesis-Related) proteins and some genes implicated in SAR. In order to understand the function of these transcription factors in grapevine, in relation with its response to pathogens, we have cloned and sequenced a cDNA encoding a putative WRKY protein (*VvWRKY2*) from a grape berry cDNA library. This gene belongs to group I in WRKY family and the deduced amino acid sequence shows high homology to *NtWRKY2* (Q9ZPL6) and *AtWRKY3* (Q9ZQ70) which are implicated in the response to pathogens. To investigate the potentiality of this factor to induce an increased resistance to phytopathogens, *Nicotania tabacum* plants have been transformed with cDNA under the control of the cauliflower mosaic virus 35S RNA promoter via *Agrobacterium tumefaciens*. The T0 transgenic plants showed an increase in the level of some pathogenesis-related genes. The response of these transgenic plants to several pathogens was studied (*Pythium* spp. and *Alternaeria tenuis*).

ISOGENE SPECIFIC OLIGO ARRAYS REVEAL MULTIFACETED CHANGES IN GENE EXPRESSION DURING GRAPE BERRY (*VITIS VINIFERA* L.) DEVELOPMENT

N. Terrier[a], D. Glissant[b], J. Grimplet[a], F. Barrieu[c], P. Abbal[a], C. Couture[c], A. Ageorges[a], R. Atanassova[b], C. Léon[c], J.P. Renaudin[c], F. Dedaldechamp[b], S. Delrot[b], S. Hamdi[c] And C. Romieu[a]

[a]*Unité mixte de Recherche S.P.O., Biologie Intégrative de la vigne et du vin, I.N.R.A., 2 Place Viala, 34060 Montpellier Cedex 1. France*
[b]*Unité mixte de Recherche C.N.R.S. 6161, Transport des Assimilats Université de Poitiers, Laboratoire de Physiologie, Biochimie et Biologie Moléculaire Végétales, 40 Avenue du Recteur Pineau, 86022 Poitiers, France*
[c]*IBVM - UMR 619, Equipe Biologie de la Vigne, Université de Bordeaux 1, 71 avenue Edouard Bourleaux, 33883 Villenave d'Ornon Cedex, France*

The transition from a green, hard and acidic pericarp to a sweet, soft, coloured and sugar rich ripe fruit is convergent in many unrelated fruit species. Ripening appears as a multifaceted developmental process involving complex pre-programmed changes in gene expression. Much remains to be learned about how these changes are initiated in non-climacteric fruits, where ripening is not accompanied by the autocatalytic emission of ethylene. Identification of differentially expressed genes has been achieved by the use of high throughput molecular approach: 50 mers oligoarrays bearing a set of 3200 unigenes from *Vitis vinifera* berries were developed and used to compare berry transcriptome at 9 developmental stages. Analysis of transcripts profiles revealed that most activations were triggered simultaneously with softening, which occurs within 24 hours only for an individual berry, just before any changes in coloration and water, sugars and acids contents were detected. Most dramatically induced genes belong to unclear functional categories. Numerous rearrangements occurred between isogenes involved in cell-wall, primary and secondary metabolism. This study of the ripening process using isogene specific oligoarrays also revealed new hormonal regulation and transcription factors as possible upstream control points of ripening in non-climacteric fruits.

COMBINING LINKAGE ANALYSIS AND LINKAGE DISEQUILIBRIUM MAPPING TO DISSECT THE GENETICS OF FRUIT COLOR IN GRAPEVINE

Christopher L. Owens

USDA-ARS, Cornell University, Geneva, NY 14456, USA

Anthocyanins are an important group of plant pigments and are the compounds responsible for the coloration of grape berries. The genetics of anthocyanin biosynthesis have been extensively studied in crop plants. In grape, several of the genes in involved in anthocyanin biosynthesis have been previously cloned and characterized but little precise information is known concerning the genetics of this important trait. Our objectives were to combine linkage analysis (i.e. QTL analysis) of anthocyanin content and composition with analysis based on linkage disequilibrium of candidate genes to dissect the genetics of fruit color in grapevine. To this end the pattern and extent of DNA sequence diversity within the genes of the grapevine anthocyanin biosynthetic pathway were determined and genetic associations between DNA sequence polymorphisms within these candidate genes and genotypes varying in fruit color were examined. Single nucleotide polymorphisms were detected in all loci examined. Results of tests of association between DNA sequence polymorphisms within the anthocyanin biosynthetic pathway and both qualitative and quantitative measures of phenotypic variation in fruit color will be discussed. Additional data on the mapping of anthocyanin candidate genes, and the QTL analysis of anthocyanin content and composition will also be presented.

GRAPEVINE ESTS AND THEIR USE IN MICROARRAY ANALYSIS TO STUDY GENES CONTROLLING GRAPEVINE BERRY RIPENING AND DEVELOPMENT

M.R. Thomas[a], C.T. Hua[a,b], P. Iocco[a,b], C. Davies[a]

[a] *CSIRO Division of Plant Industry, PO Box 350, Glen Osmond, SA 5064, Australia*
[b] *CRC for Viticulture, PO Box 154, Glen Osmond, SA, 5064, Australia*

We have constructed a range of cDNA libraries from different developmental stages during Cabernet Sauvignon grapevine flower and berry development. Expressed sequence tags (ESTs) have been characterised from these libraries and the resultant clones have been used to create a cDNA microarray containing 1368 unigenes. This array has been used in microarray analysis to investigate changes in gene expression at a global level during berry development over a two year period. In addition, we have conducted experiments looking at differences in gene expression between fruit at similar developmental stages but grown in commercial vineyards under different viticultural and/or environmental conditions. A variety of software packages have been used to analyse the resultant data including the commercial package Genespring. Our results provide interesting insights into berry development and demonstrate the complexity and problems that can arise in the production and analysis of such extensive data sets from field grown plants. Ultimately the aim of this research is to be able to understand berry development and the interaction between plant and environment at a level that will allow us to better manipulate fruit development and hence wine quality.

EFFECTS OF SOIL WATER AVAILABILITY ON THE PHYSIOLOGY OF *VITIS VINIFERA* L. (cv. PROSECCO) AND THE QUANTITATIVE, QUALITATIVE, SOCIAL AND ECONOMIC ASPECTS OF ITS PRODUCTION

Giovanni Cargnello

Director, SOC Tecniche Colturali, Istituto Sperimentale per la Viticoltura, Viale 28 Aprile 26, 31015 Conegliano (Treviso) Italy (cargnellogiovanni@libero.it)

This paper will detail original research on the production and sensory analysis of grapes to be used for the establishment of viti-vinicultural production practices within a societal context. The study utilized the 'Grande Filiera' methodology, which evaluates 54 variables within a production system including ethical, moral, environmental, socio-economic, technical and productive aspects. The study was conducted in a Prosecco vineyard subjected to either mild or severe soil water deficits. A Sylvoz trellis system was used and the vine and row spacings were 1.5 and 3.0 m, respectively. The greater water stress reduced berry and cluster weight and decreased yield approximately 50% when compared to the milder stress. At harvest the fruit of vines subjected to the greater water stress were less 'ripe' due to decreased polyphenols and aromatic characteristics, non-typical for the cultivar, in addition to unfavourable effects on soluble solids and acid. The wine had the same characteristics. It is concluded that for this location in Italy the best option for the cultivation of grapevines is a mild water stress when compared to that of a severe water stress. A mild water stress also appears to be better than more abundant water availability on fruit quality. These results also should be addressed in the context that water resources in this area of Italy are increasingly scarce and should be protected.

THE EFFECTS OF IRRIGATION AMOUNTS AND VINE SPACING ON PHYSIOLOGY, GROWTH AND PRODUCTIVITY OF TEMPRANILLO GRAPEVINES.

J. Yuste, J.L. Asenjo, MªV. Alburquerque, and J.A. Rubio

I.T.A. de Castilla y León, Ctra. Burgos, km. 119, 47071 Valladolid. Spain

Irrigation of vineyards is a matter of controversy in areas where premium wine is produced. This may be due a closer relationship between water availability than the irrigation regime itself on vine water relations. Therefore, vine spacing and the criteria used to schedule vineyard irrigations is very important. In order to better manage vineyards, information on the effects of various vineyard irrigation practices on vine physiology, productivity and fruit quality would be helpful. Therefore, a study was conducted on Tempranillo grapevines in a vineyard located in the province of Valladolid, Spain. Irrigation treatments included: 1.) No irrigation (dryland farmed), 2.) Irrigation amounts at 20% of ET_o, and 3.) Irrigation amounts at 40% of ET_o. Vine spacing treatments were 2645 vines per ha (2.20 x 1.15 m) and 3953 vines per ha (2.70 x 1.4 m). Photosynthesis, stomatal conductance, leaf water potential (? $_l$), leaf area development and productivity were measured. Photosynthesis (A) and stomatal conductance (g_s) were closely related to leaf water potential. The seasonal course of A and g_s were similar among the irrigation treatments. Photosynthesis and g were reduced to a much greater extent for vines in the non-irrigated treatment compared to vines that were irrigated. The reductions in dry matter production, leaf area and yield as a function of irrigation and vine spacing treatments were not closely related to measurements of leaf A. The measurements of A, g_s and ? $_l$ during the study were not correlated with indirect measures of whole vine physiology, estimated from measuring growth rates and yield, as a function of irrigation treatment.

INTEGRATED STRATEGIES TO MANAGE SEASONAL VARIATION IN WINE GRAPE MATURATION: DEFICIT IRRIGATION AND HEAT STRESS

N.M. Cooley, P.R. Clingeleffer and R.R. Walker

Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, SA, 5064 and CSIRO Division of Plant Industry, PMB, Merbein, Vic., 3505

In recent years several wine grape producing areas of Australia have experienced problems with grapes not attaining required sugar concentrations within reasonable time frames. Combinations of heat stress and water stress are believed to compound the delay in maturation. This project was set up to study the interaction between weather conditions and that of water stress where a control treatment (standard drip) was compared to a regulated deficit irrigation (RDI) and a prolonged deficit (PD) treatment during two seasons (2003 and 2004). The trial tests the hypothesis that stress associated with commercial RDI practices and a PD could alter fruit maturation. During the initial stress period, throughout both seasons, when temperatures were above 36°C, midday photosynthesis, transpiration and leaf water potential were significantly reduced with the deficit treatments when compared to the control. Photosynthesis and transpiration on the RDI treatment were found to recover upon return to normal irrigation, however, for the PD treatment during 2003 it took approximately 1.8 months. Alterations in trunk carbohydrate concentrations were observed with the deficit irrigation treatments during the stress period(s). The carbohydrate concentration recovered following the RDI treatment whereas reductions continued throughout the season with PD treatment during 2003. At harvest, the water deficit treatments during season 2003 were found to reduce berry size resulting in increased TSS, anthocyanin and phenolic concentration. Wine quality, when rated on density and colour, was also found to be improved with the deficit irrigation treatments compared to the control. The issue of sustainability of the RDI treatment will be discussed.

LIGHT RESPONSE OF LEAF PHOTOSYNTHESIS IN TEMPRANILLO GRAPEVINES (*VITIS VINIFERA* L.) IN TWO IRRIGATION TREATMENTS

P. Sánchez-de-Miguel, A. Centeno and J.R. Lissarrague

Departamento de Producción Vegetal. Fitotecnia., Universidad Politécnica de Madrid, Madrid, Spain

We studied the response of leaf net photosynthesis to different water regimes under Mediterranean environmental conditions (Madrid-Spain). The experiment was conducted on thirteen year old field-grown Tempranillo grapevines grafted onto 110R. Plant density was 2 m x 1.2 m. The training system was a bilateral cordon with vertical shoot positioning. Two irrigation treatments were established based on two crop coefficients; K_c = 0.2 and K_c = 0.4. Leaf area index, geometrical and ecophysiological parameters were evaluated during the ripening phase. Net photosynthesis was measured on main and lateral shoot leaves, located at the bottom, middle, upper and inner positions of the canopy, both east and west exposures. The light response of photosynthesis and leaf water potential were measured keeping leaf temperature between 24 and 30°C. Our results indicate that the light response of photosynthesis was higher for younger leaves on the main and lateral shoots compared to older leaves. The light response of photosynthesis was higher for lateral shoot leaves than for main shoot leaves, when canopy position and exposure were the same. Leaf net photosynthesis response was greater as applied water amounts increased. Differences between treatments decreased as did leaf water potential.

AGRONOMIC AND ECOPHYSIOLOGICAL RESPONSE OF FIELD-GROWN CABERNET SAUVIGNON GRAPEVINES UNDER THREE WATER MANAGEMENT REGIMES

P. Baeza, P. Junquera, J.R. Conde and J.R. Lissarrague

Departamento de Producción Vegetal. Fitotecnia., Universidad Politécnica de Madrid, 28040 Madrid. Spain

In order to develop an irrigation vineyard schedule under Mediterranean environmental conditions, a study on water management was conducted in the southeastern portion of Madrid county – Spain – at an elevation of 800 m. Cabernet Sauvignon grapevines grafted onto SO4 were used. Plant density was 2.5 m x 1.1 m. Vines were vertical shoot positioned. Three treatments were established, T1 with a constant crop coeficient (K_c) of 0.2 from the first irrigation to the end of the irrigation season, T2 with a K_c of 0.2 until veraison and a K_c of 0.45 after veraison, and T3 with a K_c of 0.45 until veraison and a K_c of 0.2 after veraison. Yield components, must composition, and ecophysiological parameters were studied. No significant differences between treatments were observed for yield components or must composition parameters. Daily evolution of net photosynthesis was higher in those treatments with higher water availability. Increasing water supply after veraison resulted in a greater efficiency of leaves expressed as SA to yield ratio or as increment of yield per each 10 mm of water supply.

COUPLING OF PLANT TO SOIL WATER STATUS IN DIFFERENT VINEYARD SITES

Bernd R. Gruber[a] and Hans R. Schultz[a,b]

[a]*Institut für Weinbau und Rebenzüchtung, Forschungsanstalt, D-65366 Geisenheim, Germany*
[b]*Fachhochschule Wiesbaden/Geisenheim, D-65366 Geisenheim, Germany*

The spatial variability in soil-type and depth and water holding capacity is very high in many viticultural regions of the world. Thus, water management needs to be site specific, and plant available soil water needs to be assessed for each vineyard or even at different positions within a single vineyard. Due to differences in rooting depths and water extraction profiles and their seasonal dynamics, only direct measurements of vine water status supply sufficient information for management decisions such as irrigation scheduling. Whole-plant transpiration rate, midday -, pre-dawn leaf - (Ψ_{PD}) and/or stem water potential have been proposed to guide these decisions, yet the coupling of these water status indicators to soil water status is problematic and usually unknown. We have used the concept of total transpirable soil water (TTSW) and the fraction thereof (FTSW), originally proposed for herbaceous plants, to evaluate the coupling between soil water availability and plant water status measurements in an irrigation trial with the cultivar White Riesling on three contrasting vineyard sites across a spatial transect of 20 km. Soil water holding capacity over the root profiles was 380, 260 and 100 L/m^2, TTSW was 175, 130, and 50 L/m^2, respectively. We found a single common relationship between Ψ_{PD} and FTSW for all vineyards, irrespective of water extraction profiles and canopy systems. This relationship was not affected by hysteresis after intermittent rainfall events. In contrast stem water potential basically reflected changes in plant hydraulic conductance as estimated from sap flow data and was largely uncoupled from soil water availability. We currently exploit using the Ψ_{PD} to FTSW relationship in a model for irrigation scheduling.

PHYSIOLOGICAL MECHANISMS INVOLVED IN THE PRODUCTION OF NON HYDRAULIC ROOT SIGNALS BY PARTIAL ROOT DRYING

Ben-Ami Bravdo

Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, POB12, Israel, 76100, email: bravdo@agri.huji.ac.il

Parallel to the progress in irrigation methods and technology during the last decades, there is a growing interest in studying physiological mechanisms of plants in order to use them as irrigation scheduling guides. Scheduling irrigation in drip systems consists of frequent irrigations applied only to part of the root system as well as non uniform soil water distribution. The dynamic nature of such systems encounters difficulties in utilization of traditional parameters such as "permanent wilting point," "field capacity" and "available water." When various parts of the root system are subjected to a wide range of soil water potentials, root to root transfer of water and minerals is an important fundament of the mechanism involved. Irrigating only a part of the root system does not change the basic pattern of root distribution, and various root types such as feeding rootlets and supporting roots are still formed according to the genetic trait of each variety. There is however an intensive development of small diameter rootlets with a large surface area and a lot of active root tips in the soil beneath the drippers. Such intensive rootlet system enhances uptake of water and minerals, as well as intensive production of growth regulators (PGR) operating as root signals. Cytokinins, abscisic acid (ABA) and gibberellins are the major PGR formed in the root tips and their partition into various parts of the canopy is involved in various physiological processes. The ability to control the soil water potential of various parts of the root system by different types of microirrigation systems led researchers to try and affect the production of root signals and thereby increase water use efficiency, as well as canopy architecture and fruit bud differentiation. A method of partial root drying (PRD), where irrigation is alternatively applied every two weeks to one of the two sides of the plant, was reported to increase ABA production, reduce cytokinin production, and raise the xylem pH by drying roots. A non-hydraulic regulation of stomatal conductance, shoot, leaf and fruit growth as well as increased water use efficiency, was measured in split root experiments conducted in containers or root restricting compartments. Such results have never been reported for PRD open field experiments. It may well be that an absolute control of root drying is essential for obtaining such non-hydraulic effects and drip irrigation under field conditions does not provide means for drying 50% of the roots and inducing concomitant non-hydraulic effects.

WATER DEFICITS, YIELD, AND BERRY SIZE AS FACTORS OF COMPOSITION AND WINE SENSORY ATTRIBUTES

Mark Matthews

Department of Viticulture & Enology, University of California, One Shields Avenue, Davis, CA 95616-8749 USA

Crop yield and berry size are widely recognized as important factors in the quality of resultant wines. However, most prior research has shown no effect of yield on wine quality, and the direct effect of berry size has not been evaluated. Berries from Cabernet Sauvignon vines that had experienced High, Control, and Low water statuses during ripening were segregated into 6 size categories at harvest in order to test independently for relationships due to size compared with those due to water deficits. Water deficits increased the relative seed and relative skin mass. Differences in relative seed and skin mass due to water status were greater than those associated with berry size. Content of all solutes increased approximately in proportion to berry size. Deviations from proportionality caused °Brix and anthocyanin concentration to decrease, and the concentration of skin tannin to remain unchanged with increasing berry size. The results show that there are effects of water status on fruit composition that arise independent of the differences in fruit size. Wines made from the Low treatment were significantly higher in fruity aromas and fruit by mouth than the wines from the irrigated treatments. The role of yield in the sensory properties was tested using pruning and cluster thinning to manipulate yield, creating yields that varied from 4.3 to 22.2 t ha^{-1}. Wines made from vines pruned to low yield were higher in vegetal aroma and flavor, bell pepper aroma, bitterness, and astringency than 'high yield' wines. In contrast, there were few sensory differences detected in wines made from the various cluster-thinning treatments. 2-methoxy-3-isobutylpyrazine (MIBP) concentrations were significantly negatively correlated with buds per vine. In addition, MIBP concentration was directly related to sensory vegetal intensity ratings obtained by descriptive analysis. Thus, Cabernet Sauvignon aromas and flavors respond to yield manipulation, but do so significantly only when yield is altered early in fruit development.

VINE WATER RELATIONS AND PRODUCTIVITY OF FIELD-GROWN *VITIS VINIFERA* L. IN RESPONSE TO HIGH FREQUENCY DRIP IRRIGATION

L.E. Williams

Department of Viticulture and Enology, University of California – Davis and Kearney Agricultural Center, Parlier, CA 93648

A field study was conducted on *Vitis vinifera* L. (cv. Thompson Seedless) to evaluate various measurements of vine water status under high frequency drip irrigation. Water use at 100% of vine evapotranspiration (ET_c) was determined with a weighing lysimeter. Vines in the surrounding vineyard were irrigated at 0, 0.2, 0.6, 1.0 or 1.4 times the amount of water used by the lysimeter vines. Water applications occurred each time the lysimeter lost 16 L of water (2 mm depth; 8 L $vine^{-1}$). Soil water content (θ_v) was measured in the 0.2, 0.6, 1.0 and 1.4 irrigation treatments. Pre-dawn (Ψ_{PD}), midday leaf (Ψ_l) and midday stem (? $_{stem}$) water potentials were measured at the ends the 1991 and 1992 growing seasons and almost monthly during 1993. Soil water content in 1993 remained constant throughout the growing season for the 1.0-irrigation treatment; it increased in the 1.4-treatment and it decreased in the 0.2 and 0.6-treatments. Both Ψ_l and Ψ_{stem} measurements detected differences among irrigation treatments to a greater extent than did Ψ_{PD} until very late in the 1993-growing season. There was a linear relationship between Ψ_l and Ψ_{stem}. All three measurements of water potential were related to soil water content (using a quadratic function) however, the relationship between SWC and Ψ_{PD} had the lowest r^2 value, 0.52 compared to 0.90 and 0.94 for Ψ_l and Ψ_{stem}, respectively. A reduction in yield and pruning weights from 40 to 60% was measured when Ψ_{PD} decreased from -0.05 to -0.15 MPa. The results indicated that Ψ_{PD} would not be useful in accurately determining vine water status or its effects on productivity under high frequency deficit-irrigation.

THE REGULATION OF POLYGALACTURONASE INHIBITING PROTEINS IN GRAPEVINE (*VITIS VINIFERA*)

D.A. Joubert[a], M.A. Vivier[a], G. de Lorenzo[b] and I.S. Pretorius[c]

[a]*Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, Stellenbosch, South Africa*
[b]*Dipartimento di Biológia Vegetale, Università di Roma "La Sapienza, Roma, Italy*
[c]*The Australian Wine Research Institute, Adelaide, Australia*

Polygalacturonase inhibiting proteins (PGIPs) are cell wall proteins that specifically inhibit various fungal polygalacturonases (PGs) and are involved in plant defense responses. PGIPs are encoded by small gene families, and the inhibition profile of each individual PGIP within a family is often unique. The expression of PGIPs is regulated by various stimuli, including physiological, environmental and pathogen related factors. Recently, a PGIP encoding gene from grapevine (*Vvpgip1*) has been cloned in our laboratory. Expression of PGIP in grapevine is developmentally regulated, is berry-specific and is induced in a tissue-independent manner by wounding, osmotic stress, *Botrytis cinerea* infection, indole acetic acid and salicylic acid. In addition, expression is down regulated by a staurosporine-sensitive protein kinase, suggesting the involvement of protein phosphorylation in the signal transduction cascade that leads to PGIP expression. PGIP induced by *B. cinerea* infection, wounding and osmotic stress in leaves displayed the same PG inhibition spectrum as that of the product of the cloned *Vvpgip1* gene. The mRNA induction profile of grapevine PGIP was mimicked in transgenic tobacco expressing the cloned *Vvpgip1* gene under control of its own promoter, indicating that regulatory mechanisms for PGIP expression are conserved in tobacco and grapevine. Also, using PCR generated promoter deletions, promoter areas involved in auxin- and *Botrytis* responsiveness were mapped to the area between positions –3.1 kb and –1.5 kb. Responses to osmotic stress (novel to *Vvpgip1*) involve the area between positions –1.1 kb and -0.4 kb while wound responses are mediated by the area between positions –0.4 kb and –0.1 kb.

OVEREXPRESSION OF THE *VITIS VINIFERA* L. β–CAROTENE HYDROXYLASE GENE IMPROVES THE PHOTOPROTECTIVE ABILITY OF TOBACCO

Philip R. Young[a], Melané A. Vivier[a], Shang W. Chen[b] and Isak S. Pretorius[c]

[a]*Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, Stellenbosch, South Africa*
[b]*Food Science and Nutritional Engineering College, China Agricultural University, Beijing, Peoples Republic of China*
[c]*Australian Wine Research Institute, Adelaide, Australia*

Every year severe crop reductions are caused by extreme environmental conditions that typically cause oxidative damage in plants. Carotenoids associated with the photosynthetic membranes serve a vital protective function under these conditions, and are proving a promising target for manipulation in order to enhance the plants' inherent stress tolerance. To this end, the cDNA- and genomic copies of the β–carotene hydroxylase (BCH) encoding gene from *Vitis vinifera* has been isolated and characterized. The BCH gene contains six introns and is present as a single-copy gene in the grapevine genome. The BCH gene is expressed at relatively low-levels in leaves, berries, and flowers. A 2.0 kb fragment of the putative BCH promoter showed no transcriptional activity in transient reporter gene assays. *Nicotiana tabacum* was transformed with the BCH-encoding gene from grapevine which was constitutively overexpressed in both the sense and antisense orientation. Under mild photon flux densities (200-400 µmol m^{-2} s^{-1}) the T_1-generation expressing the BCH gene were phenotypically indiscernible from untransformed plants, and showed no differences in either growth rate or total carotenoid content. Under high light intensities (1500-2500 µmol m^{-2} s^{-1}), however, the photosynthetic parameters of the transgenic tobacco lines showed significant differences relative to both the control and antisense plants. Chlorophyll *a* fluorescence assays revealed that the expressed BCH assisted the transgenic plants in coping with high light stress by reducing photodamage to the PSII reaction centre while maintaining a lower D1 protein turnover rate, as well as a higher net assimilation rate. The rate of recovery and relaxation of the active reaction centers of the transformants during the dark phase was higher than that of the control plants. During the illumination phase the transgenic plants displayed slightly elevated xanthophyll pigment pools in leaves and managed to maintain higher stomatal conductance. Cumulatively, these results indicate that the expression of the grapevine BCH functions in transgenic tobacco by enhancing the plants existing photoprotective system under high light stress. This can be accounted for by the availability of additional zeaxanthin in the xanthophyll biosynthetic pathway.

CHARACTERIZATION OF TWO TRANSCRIPTION FACTORS INVOLVED IN THE REGULATION OF PHENYLPROPANOID METABOLISM IN GRAPE BERRY

L.G. Deluc[a], C. Marchive[a], F. Barrieu[a], A. Descendit[b], J.M. Mérillon[b], and S. Hamdi[a]

[a]*UMR PBV – IBVM, Equipe Biologie de la Vigne – Université de Bordeaux I, BP81, F-33883 Villenave d'Ornon, France.*

[b]*Laboratoire de Mycologie et biotechnologie Végétale, EA 491, UFR Pharmacie, Université Victor Segualen Bordeaux 2, 146, rue Léo Saignat, 33076 Bordeaux cedex,*

Despite the importance of polyphenols on wine quality, very little is known about the regulation of genes involved in polyphenol biosynthesis in grape berries. One of the main goals of our laboratory is the identification of molecular markers associated with polyphenol content of grape berries during development. Such markers may be helpful in understanding how grapes ripen and how their quality evolve during ripening to optimize harvest dates and subsequent processing. In plants, expression of genes involved in polyphenols biosynthesis appears to be regulated by at least two families of transcription factors (MYB and bHLH). By PCR-based screening of grape berries cDNA libraries, we have isolated four cDNA clones encoding putative Myb proteins (VvMybCs1, 2, 3, and 4). Expression of VvMybCs2 is very high during the early stages of berry development and decreases just before véraison whereas the expression of VvMybCs4 is very low during the early stages and greatly increases at the onset of berry ripening. Finally, VvMybCs2 and VvMybCs4 appear not to be specific fruit genes as expression of both genes can be detected in leaves, shoots and roots. To investigate *in vivo* function of VvMyb genes, we generated transgenic tobacco plants constitutively expressing VvMybCs2 or VvMybCs4 cDNAs. Analysis of over expressing plants indicates that both VvMybCs2 and 4 triggered a strong accumulation of anthocyanins in tobacco flowers. Semi-quantitative RT-PCR analysis indicated that over expression of VvMybCs2 and VvMybCs4 in transgenic plants correlates with an enhanced expression of numerous genes involved not only in anthocyanins but also in monolignols biosynthesis. Taken together, our results indicate that VvMybCs2and VvMybCs4are involved in the regulation of phenylpropanoid metabolism in grape berries. Because of the possible implication of monolignols in berry defense against pathogens, we are currently investigating the resistance of transgenic tobacco over expressing VvMybCs2 and VvMybCs4 to various pathogens.

STRESS- AND TISSUE-SPECIFIC EXPRESSION OF CBF GENES IN GRAPE

Huogen Xiao[a], Elizabeth A.R. Tattersall[b], Siobhan Braybrook[a], Grant R. Cramer[b] and Annette Nassuth[a]

[a]*Department of Botany, University of Guelph, Guelph, ON, Canada*
[b]*Department of Biochemistry, University of Nevada, Reno, NV, USA*

It has been reported that CRT-binding transcription factors (CBFs), also called drought responsive element binding proteins (DREBs), activate a pathway that leads to increased freezing and drought tolerance in *Arabidopsis thaliana*. A trigger such as a cold period appears to be required to activate transcription of the *AtCBF* genes. This paper reports on the identification of *CBF* genes and their transcripts in grapes. Four different CBF genes were identified and isolated from both the freezing tolerant *V. riparia* and the freezing susceptible *V. vinifera*. The deduced amino acid sequences were highly identical (96-99%) between *V. riparia* and *V. vinifera* homologs and all contained the AP2 region and signature sequences expected for CBF proteins. Three of the *Vitis* CBF sequences were more similar to each other (62-69%) than to the fourth (44-48%). The similarity of the four *Vitis* CBF genes to AtCBF1 was between 42 to 58 %. *CBF1* and *CBF3* transcripts were detected in apical tips, leaves, buds and stems, and *CBF4* transcripts in leaves and buds of young shoots growing under control conditions. Cold treatment increased transcript levels for *CBF1, 2* and 3 in leaves and stems, and for *CBF4* in leaves, stems and buds. Older leaves plants grown under control conditions did not appear to have any *CBF* transcripts but cold induced in these leaves transcripts of *CBF4*, and to a lesser extent of *CBF3*. Transient expression experiments in tobacco leaves showed that both CBF1 and CBF4 can induce expression of the reporter gene *GFP* with a RD29A promoter, which contains a CRT/DRE element, supporting a role for grape CBF proteins in stress tolerance.

MOLECULAR ANALYSIS OF WATER STRESS EFFECTS ON AQUAPORIN GENES EXPRESSION IN DIFFERENT *VITIS VINIFERA* CULTIVARS

R. J-B. Fouquet[a], F. Barrieu[a], N. Ollat[b], and S. Hamdi[a]

[a] *UMR PBV – IBVM, Equipe Biologie de la Vigne – Université de Bordeaux I, BP81, F-33883 Villenave d'Ornon, France*
[b] *Unité de recherches sur les Espèces fruitières et la Vigne, INRA, CR de Bordeaux, BP81, F-33883 Villenave d'Ornon, France*

Water uptake is a critical process for plant growth. In grapevine, the capacity to develop an extensive root system in order to enhance water uptake is an important component of the plant vigor. In most cases, strongly vigorous *Vitis* species appear to be more tolerant to drought. Aquaporins are membrane water channels that play critical roles in controlling the water content of cells and tissues. In plants, aquaporins are found in the plasma and vacuolar membranes. Because of the key role of aquaporins in the control of water uptake, the possible relation between aquaporins and drought resistance in different grapevine cultivars was investigated. To identify genes encoding aquaporins in grapevine, a PCR-based screening of grape berries cDNA libraries was initiated. Ten full-length cDNA encoding putative grape aquaporins have been isolated and characterized. According to database search and sequence homologies, 3 cDNAs encode putative tonoplast aquaporins (TIP) and 7 cDNAs encode putative plasma membrane aquaporins (PIP). Because of the strong homologies found within the coding sequences, specific probes representing the 3' untranslated regions of each cDNA were generated by PCR and used for the preparation of cDNA macroarray filters. To analyse aquaporin gene expression in roots of several *Vitis* cultivars, plants were grown in hydroponic conditions in the absence (non stressed) or the presence of PEG (water stressed plants). Root tissues were harvested at different times and used for the preparation of cDNA probes for macroarray hybridisation experiments. Preliminary results indicate that aquaporin gene expression patterns are significantly different in the cultivars tested in non-stressed conditions. In water-stressed plants, a general decrease of aquaporin gene expression can be observed in all cultivars but at various levels. In order to link aquaporin gene expression and water stress tolerance, future work will focus on genes differentially expressed between the *Vitis* cultivars studied.

IDENTIFICATION AND CHARACTERIZATION OF cDNAs INVOLVED IN THE TOLERANCE OF GRAPEVINE TO DROUGHT AND SALINITY

M. Hanana[a], R. Fouquet[b], S. Daldoul[a], L. Deluc[b], A. Mliki[a], A. Ghorbel[a], F. Barrieu[b] and S. Hamdi[b]

[a]*Laboratoire de Physiologie Moléculaire de la Vigne, INRST, BP95, 2050 Hammam-Lif, Tunisia, e-mail: a.ghorbel@inrst.rnrt.tn*
[b]*Laboratoire de Génomique Fonctionnelle et de la qualité de la Baie de Raisin, UMR 619 PBV, Université Bordeaux1, 71, Av. Edouard Bourleaux BP 81, 33883 Villenave d'Ornon, France, e-mail : said.hamdi@ bordeaux.inra.fr*

Our physiological investigations on grapevine tolerance to drought and salinity revealed that tolerant varieties are able to exclude Na^- and Cl^- ions into the roots and preserve their turgescence, suggesting the existence of an efficient system of osmotic regulation. In this respect, we developed a candidate gene approach to identify genes involved in these mechanisms of ionic exclusion and turgor control in grape. To date, we have isolated three cDNA encoding Na^+/H^+ antiporter, RD22 and dehydrin proteins from Cabernet Sauvignon. In general, the Na^+/H^+ antiporter is involved in the transport of sodium and hydrogen ions across the vacuolar membrane and represents a key transporter in maintaining the pH of actively metabolising cells. RD correspond to genes that are responsive to dehydration or ABA. Dehydrins are a family of plant proteins induced in response to environmental stresses such as water stress, salinity and freezing or which occur during the late stages of embryogenesis.The physico-chemical and structural properties of the putative proteins deriving from these cDNA have been characterized. The structural parameters, the conserved domains and the post-translational modification sites identified are of great importance in understanding the function of these proteins. The overexpression of the corresponding genes will be done through genetic transformation in order to study and validate their putative function.

MOLECULAR ASPECTS OF SUGAR TRANSPORT, SUGAR SENSING AND DEFENCE IN GRAPE

A. Agasse, B. Cakir, D. Glissant, A. Saumonneau, F. Dédaldéchamp, E. Gomès, P. Coutos-Thévenot, R. Atanassova and S. Delrot

Unité Mixte de Recherches, Centre National de la Recherche Scientifique 6161, Université de Poitiers, Bâtiment Botanique, 40 Avenue du Recteur Pineau, 86022 Poitiers Cédex, France

Targetted (cloning and functional studies) and non-targetted (macroarrays) approaches are currently being applied by our group to understand the molecular basis of sugar accumulation, and of defense reactions in grape. Following the cloning of VvHT1, a putative hexose transporter homologue from grape berries (Fillion et al., 1999), we showed that the expression of this gene is under the control of sugars (Atanassova et al., 2003). Using a one hybrid technique, we cloned an ASR (Abscisic acid Stress Ripening induced) protein that was able to bind to the sugar boxes present in the VvHT1 promoter, and demonstrated that the ASR protein, localized in the nucleus, controlled the expression of VvHT1. The expression of this ASR (called VvMSA) is itself under the control of sugars and ABA (Cakir et al., 2003). Over- or anti-sense expression of VvHT1 in tobacco altered assimilate partitioning, but whether VvHT1 is a sugar transporter or a sensor remains unknown (Leterrier et al., 2003). Current efforts aim at deciphering the transduction pathways involved in the sugar control of VvHT1, testing the function of VvHT1 and other transporter homologues cloned from grape berry, and obtaining an integrated pattern of expression of these genes throughout ripening by use of microarrays (see also paper by Terrier et al., this meeting). With regard to defense reactions, our main interest concerns the study of Lipid Transfer Proteins (LTP), which are strongly induced by a *Botrytis* elicitor in grape suspension cells (Gomes et al., 2002), but whose exact function is still unknown. We showed that LTP expression is also induced by sugar. An analysis run with a first generation of macroarray (3200 genes) allowed the identification of a very limited number of genes that were induced by *Botrytis* in intact berries.

MOLECULAR ECOLOGY, POPULATION GENETICS AND EVOLUTIONARY SYSTEMATICS OF WILD GRAPE SPECIES: RELEVANCE FOR GRAPEVINE PHYSIOLOGY AND BIOTECHNOLOGY

Heidi R. Schwaninger and Charles Simon

U.S. Department of Agriculture-Agricultural Research Service, Plant Genetic Resources Unit, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA

The extent of intra- and inter-specific variation of neutral or economically important traits is poorly known in wild grapes. Yet this knowledge is essential for effective in situ or ex situ germplasm preservation and its use by breeders or biotechnology. Much research is focused on *V. vinifera* but cultivar improvements through multiple means and meeting future adaptive challenges may depend on the genetic wealth held in the wild species of the genus. Wild resources will progressively decrease if conservation efforts are not made. A research program is outlined that has the long-term goals to 1) broadly study genetic variation in the genus by elucidating relationships among the species; 2) study intra-specific variation by characterizing population structure of grape species native to the United States (and other countries as available), 3) study intra- and inter-specific variation in traits of ecological, economical and biological interest, 4) improve the repository holdings of grape genetic resources to include the broadest possible spectrum of non-redundant neutral and functional genetic variation present in the genus in general and U.S. species in particular for future use in research and breeding, 5) collect information applicable toward designing effective in-situ conservation programs. Some very preliminary data will be presented.

RELIABILITY OF CLIMATE CHANGE IMPACT ASSESSMENTS FOR VITICULTURE

Manfred Stock, Friedrich-W. Gerstengarbe and Peter C. Werner

Potsdam Institute for Climate Impact Research, D-14412 Potsdam, Germany

Current assessments of climate change effects on viticulture are primarily based on results of global climate models. With respect to temperature and indices based on it this might be quite correct. Recent studies in this regard indicate that several viticultural regions will become more and others less viable as high quality wine producers (Jones 2003, Stock 2003). On the other hand we know that the allocation of chances and risks depends not only on temperature but also on a variety of other climate parameters and on climate variability. Global model resolutions are insufficient to deliver reliable results with respect to impact assessments related to precipitation, for example. Current methods of regionalization by statistical downscaling or embedded regional climate models show deficiencies and uncertainties as well. A third method for the evaluation of regional climate scenarios will be presented which shows improved reliability concerning viticultural aspects. Long lasting regional climate data records with high spatial and temporal resolution are evaluated and used as input for a simulation of future regional climate scenarios. The model, based on a special cluster and Monte-Carlo algorithm, further uses temperature change from global model calculations as a control variable. The resulting regional climate scenarios comprise meteorologically consistent sets of temperatures and associated climate parameters with daily resolution for selected viticultural regions. Improvements in reliability as well as remaining uncertainties will be discussed with respect to viticulturally relevant assessments. This method aims primarily at evaluating measures of adaptation rather than at simple predictions.

CLIMATE CHANGE IN THE WESTERN UNITED STATES GRAPE GROWING REGIONS

Gregory V. Jones, Ph.D.

Geography Department, Southern Oregon University, 1250 Siskiyou Blvd, Ashland, OR 97520, EMAIL: gjones@sou.edu

Understanding climate change and the potential impacts on natural and human-based systems has become increasingly important as changing levels of greenhouse gases and alterations in earth surface characteristics bring about planetary energy, temperature, and hydrologic changes. Observed trends and potential changes in temperatures exert strong influences on virtually every form of agriculture where production viability may be altered due to changes in winter hardening potential, frost occurrence, growing season lengths, and heat accumulation for ripening potential. The importance of understanding climate change impacts on agriculture is never more evident than with viticulture where many years of experience has resulted in the finest wines being made from grapes grown in geographically distinct regions. However, grapevines are generally grown in regions and under conditions that are considered narrow for a specific variety's optimum quality, ultimately putting it at a greater potential risk from climatic variations and change. Therefore, this research examines trends from 1948-2002 in annual and seasonal frost frequencies, the dates of last spring and first fall frost occurrence, the length of the frost-free period, and growing season average temperatures and degree-days for the principal grape growing regions in California, Oregon, and Washington. Results reveal that, on average, most regions have experienced a decline in frost frequency, earlier last spring frosts, later first fall frosts, longer frost-free periods, and warmer growing seasons with greater heat accumulation. An examination of possible future climate change in these same regions indicate an average growing season warming of 2°C in the next 50 years. The magnitude of the projected changes will potentially bring about geographical shifts in production viability or cause growers and winemakers to consider varietal or management adaptations to maintain current wine styles and quality.

CLIMATE WARMING: CONSEQUENCES FOR GENERAL VITICULTURE AND THE NOTION OF 'TERROIRS' IN EUROPE

Bernard Seguin and Inaki Garcia de Cortazar

Unite Agroclim, INRA, Site Agroparc, domaine St-Paul, F-84914, Avignon cedex 9, France

As for any crop, the impact of an anthropogenic greenhouse effect will include the stimulating effect of increased CO_2 concentration on photosynthesis, which will result in increased dry matter production and may lead to noticeable changes in cultural practices. However, it is more likely that the most significant impact will result from rising temperature, even if other climatic variables, such as rainfall also are considered. Apart from a significant displacement of the traditional limits for grapevine cultivation, serious questions may arise concerning 'terroirs': Will it be possible to keep the same cultivars by adjusting vineyard cultural and enological practices? How might viticulture and wine-making respond to the predicted increase in surface temperature? The warming trend of the last fifteen years in most of Western Europe and especially in France may provide some clues. The phenology of the grapevine has significantly advanced; one to two weeks for flowering and almost one month for harvest date in the last 50 years. The advance in harvest date also has been accompanied by changes in sugar and acid concentrations. Expressed in terms of the Huglin index, the increase in temperatures due to global warming will lead to vintages that are more uniform across years. However, there may be a tendency in which the climatic variables of a particular grape growing region will exceed the established limits for grape cultivars strongly associated with that location (terroir). The 2003 growing-season, characterized by very hot weather and drought (this being similar to the end of century climatic scenarios) provides evidence that bioclimatic indices do not take into account the possible natural adaptation of some grape cultivars to the predicted changes in climate. Therefore, only the use of more sophisticated tools such as accurate crop models, currently under development, may provide a more valuable perspective on future viticulture.

MODELING THE EFFECT OF CLIMATE CHANGE ON GRAPEVINE WATER RELATIONS

Hans R. Schultz[a,b] and Eric Lebon[c]

[a] *Institut für Weinbau und Rebenzüchtung, Forschungsanstalt*
[b] *Fachhochschule Wiesbaden/Geisenheim, D-65366 Geisenheim, Germany*
[c] *UMR Écophysiologie des plantes, sous stress environmentaux, LEPSE, INRA-ENSAM, F-34060 Montpellier, France*

According to the Intergovernmental panel on climate change (IPCC 2001), global warming will increase air temperature in Western and Northern Europe between 2.5 and 4.5 °C by the end of this century. Most climate models predict an increase in precipitation rates during winter as a result of the temperature-driven increase in the velocity of the hydrological cycle over Europe. Results are less clear for the vegetation period but dryer conditions are more likely. Higher temperatures will cause higher rates of evaporation, both from soil and from plants. Since grapevines are mostly not irrigated in Europe, there may be a substantial risk in terms of more frequent and more severe droughts with possible adverse effects on yield and quality. To assess these possible effects, we developed a model capable of simulating vine responses to drought. For this a geometrical canopy model describing radiation absorption and partitioning between grapevines and soil was coupled to a soil water balance routine describing a bilinear change in relative transpiration rate as a function of the fraction of soil transpirable water (FTSW). The model can account for changes in soil evaporation after precipitation events and has previously been validated. We used several data sets from vineyard sites differing largely in the amount of total transpirable soil water and simulated different climatic scenarios. Through a relationship between FTSW and pre-dawn water potential (Ψ_{PD}), which has proven to be stable over large differences in soil water storage capacity from different vineyard sites, we were able to simulate climate change effects on Ψ_{PD} and consequently its effects on photosynthesis and stomatal conductance.

PHYSIOLOGICAL RESPONSES OF MINIMAL PRUNING SYSTEMS TO GIBBERELLIC ACID

Karsten Weyand[a] and Hans R. Schultz[a,b]

[a]*Institut für Weinbau und Rebenzüchtung, Forschungsanstalt, D-65366 Geisenheim*
[b]*Fachhochschule Wiesbaden/Geisenheim, D-65366 Geisenheim, Germany*

Minimal pruning (MP) systems have been used successfully in Australia for more than 2 decades. These systems are highly economic and have proven to be less susceptible to bunch rot and to induce small berries with high amounts of aromatic precursors even in cool climate viticulture. However, high yields combined with an increase in water consumption due to increased leaf area can substantially delay ripening in dry years. Since mechanical thinning was unsuccessful, we started to use gibberellic acid (GA) and in some cases ethephon to reduce yield by increasing berry shatter, to modify cluster and berry appearance and to influence shoot fertility. Field experiments were performed in Geisenheim, Germany with minimal pruned *Vitis vinifera* L. cv. Riesling in 2001 – 2003. GA was applied at a concentration of 50 ppm 3 weeks after bud break (2003) and at full bloom (2002, 2003). Some treatments were localized to selectively affect certain areas within the canopy (2003). In contrast to pruned vines, MP vines did not respond to bloom time applications of GA with a reduction in yield but sugar concentration decreased. The early GA application reduced yield up to 20%, but did not increase sugar concentration. We found that GA treatments caused pre-dawn leaf water potential to remain less negative for treated vines as compared to the control until harvest. Leaf gas exchange measurements showed that GA application induced a reduction in photosynthesis (PS) and stomatal conductance, thus transpiration rate, which may have caused the effect on leaf water potential and berry sugar concentration. This effect on PS was persistent and in some cases carried over to the next season.

HYDRAULIC ARCHITECTURE, CAVITATION SUSCEPTIBILITY AND GAS-EXCHANGE OF SEVERAL GRAPEVINE VARIETIES OF DIFFERENT GEOGRAPHIC ORIGIN

Antigone Chouzouri[a] and Hans R. Schultz[a,b]

[a]*Institut für Weinbau und Rebenzüchtung, Forschungsanstalt, D-65366 Geisenheim, Germany*
[b]*Fachhochschule Wiesbaden/Geisenheim, D-65366 Geisenheim, Germany*

Previous research about varietal strategies in response to water deficits indicated that differences in hydraulic architecture may account for differences in stomatal sensitivity. We investigated the relationships between hydraulic conductance (Kh) in stems and petioles (measured with the pressure chamber), susceptibility for embolism formation in the xylem (detected by ultra-sound acoustic emission signals, AE's) and stomatal responsiveness (inferred from gas-exchange measurements) to a developing water deficit in both pot and field studies. The study was focused on four genotypes, typical of different growing areas in Europe largely varying in water supply; Airen (Spain), Grenache (Spain/France), Syrah (France), Silvaner (Germany). The decline in stomatal conductance occurred concomitant to the increase in AE's for all varieties, but at very different absolute emission rates. Silvaner and Airen were the most sensitive to xylem embolism formation in the stem during the initial dry down phase in a pot experiment. After reaching a maximum, AE's declined again until stomatal closure was complete in Silvaner and Airen but not in Grenache and Syrah. Hydraulic conductance of internode segments was highest for Airen and Silvaner, followed by Grenache and Syrah. For example at a plastochron index of 20 (~equal to node position from the shoot tip), Silvaner had a fourfold higher conductance than Syrah in the field. When stomatal conductance approached zero, Syrah had exploited soil available water the most, and Silvaner and Airen the least. It seems that strategies in water use are correlated with plant architecture.

CHANGES IN THE EVOLUTION OF MUST COMPOSITION DURING RIPENING OF TEMPRANILLO GRAPES (*VITIS VINIFERA* L.) IN TWO IRRIGATION TREATMENTS

A. Centeno, P. Sánchez-de-Miguel, R. Linares, and J.R. Lissarrague

Departamento de Producción Vegetal: Fitotecnia, Universidad Politécnica de Madrid, Madrid, Spain

Must composition (Tempranillo grapevines grafted onto 110R) as affected by water availability was studied during the 2002 and 2003 growing seasons. The study was conducted in Madrid, Spain, at an elevation of 600 m above sea level. Vines were vertical shoot positioned with a 2 m x 1.2 m plant density. Two irrigation treatments were established based on two crop coefficients, $K_c = 0.2$ and $K_c = 0.4$. Irrigation was initiated when shoot elongation rate began to decrease. Changes in berry weight, total soluble solids, titratable acidity, pH and phenolic compounds were determined weekly from veraison to harvest. We will present leaf area index and yield components for both treatments. We compared leaf net photosyntesis on reference adult leaves between treatments. No significant differences between treatments were obtained in must composition, and hence on grape juice quality; nevertheless, higher yield was observed for the treatment in which greater amounts of water were supplied.

SEASONAL EVOLUTION OF THE SOIL-WATER PROFILE AND LEAF PRE-DAWN WATER POTENTIAL IN FIELD-GROWN GRAPEVINES SUBJECTED TO DIFFERENT WATER REGIMES

E. Cuevas[a], P. Baeza[b] and J.R. Lissarrague[b]

[a]*Département Productions Agricoles, ENITA de Bordeaux, Gradignan, France*
[b]*Dpto. de Producción Vegetal: Fitotecnia, Universidad Politécnica de Madrid, Madrid, Spain*

The influence of irrigation regime on the seasonal evolution of soil water content was studied in field-grown grapevines (*Vitis* vinifera L.) under semi-arid conditions. The four irrigation regimes examined were no-irrigation and crop coefficients (K_c) of 0.15, 0.30 and 0.45 with water applied by drip irrigation. Pruning was adjusted to water availability according to a balanced approach. Soil-water content (θ_v) was measured weekly at four depths (0-20, 20-40, 40-70 and 70-110 cm) using Time Domain Reflectometry (TDR). Soil-water depletion levels up to a depth of 110 cm confirmed that seasonal vine ET surpassed the volume of water supplied to the different experimental treatments. The lowest soil water levels were measured at the 40-70 cm depth, where differences in θ_v among water regimes were the highest. At the end of the season levels of θ_v at this depth reflected the volumes of water applied, with the exception of the 0.15 K_c treatment. For that treatment, soil water depletion during maturation was even more intense than for the non-irrigated treatment. When comparing the three irrigation treatments it was observed that increasing irrigation volumes resulted in superior soil water levels in depth, parallelly, higher number of fruiting shoots where maintained. Irrigation, even at the lowest level (K_c = 0.15) mimicked the seasonal pattern of pre-dawn leaf water potential. For the non-irrigated treatment, soil water depletion by depth was maximum at 40-70 cm, followed by the 20-40 cm interval, and lastly at the 70-110 cm depth. For this treatment, soil water depletion by depth-interval would have corresponded well with soil-water extraction by roots at that depth, given the scarcity of rain during summer.

SOIL CHARACTERISTICS AND WATER CONTENT OF VINEYARDS UNDER NON-IRRIGATED CONDITIONS: VITICULTURAL AND OENOLOGICAL RESULTS FOR SANGIOVESE GRAPEVINES

P. Storchi, E.A.C. Costantini and P. Bucelli

Istituto Sperimentale per la Viticoltura, Conegliano (Italy), storchi@ispervit.it

A study was conducted in the Province of Siena (central Italy), where some of the most renowned DOCG wines of Italy are produced. The study was conducted to characterize important soil qualities, which may affect vine growth and wine quality. Seven meteorological stations were used to characterize the climate of the grape-growing region. The results of a set of 10 experimental plots with the cultivar "Sangiovese," located in 7 vineyards during 2002 and 2003 were utilized to obtain a range of soil characteristics. Climate did not differ among experimental sites, but soil characteristics such as available water capacity, drainage and rooting depth, varied greatly. Analysis of total acidity, pH, and soluble solids was determined from veraison to harvest. Cluster numbers, yield and vegetative vigour were measured at harvest time. The physico-chemical and organoleptic analyses of 20 wines made for made for the 2002-2003 vintage were carried out. High soil water content from veraison to harvest produced greater vegetative growth, delayed ripening, and reduced the sugar and phenols content of the berries. Soil with moderate water capacity and summer water stress produced better quality grapes.

WATER CONSUMPTION OF GRAPEVINES (cv. SUPERIOR) GROWN IN A SEMI ARID REGION

Y. Netzer, Y. Chongren, M. Shenker, B. Bravdo, and A. Schwartz

The Robert H. Smith Institue for Plant Sciences & Genetics in Agriculture, Faculty of Agriculture, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, 76100 Israel

Water use of *Vitis vinifera* L. grapevines (cv. 'Superior') trained as a double canopy gable system, was measured in lysimeters for five growing seasons (1999-2003) in a semi arid region in the southern part of Israel. Twelve drainage lysimeters each with a volume of 1.6 m^3 (one vine per lysimeter) were installed as part of a 1 hectare vineyard. The vines were irrigated with 4 to 8 drip emitters of 2.4 L h^{-1}. The volume of water that was supplied every day exceeded vine water consumption by at least 20%. The daily water consumption that was measured in the lysimeters served as reference for an irrigation experiment conducted in the remainder of the vineyard. Seasonal crop evapotranspiration (ET_c) curves are presented as well as reference evapotranspiration (ET_o) derived from the FAO Penman-Monteith equation. Maximum ET_c ranged from 7.6 to 8.3 mm day^{-1} for the 5 years of the study (based on an area of 6.99 m^2 per vine). Seasonal ET_c (Days 105-278) was 871 mm in 2000 and ranged from 1025 to 1081 mm the other 4 years. Crop coefficient (K_c) curves were calculated from ET_c and ET_o. A similar pattern of the seasonal K_c was obtained each year, with exception of the 2000 season where the K_c was lower due to technical problems of the irrigation system. Monthly leaf area index (LAI) was measured the last two years of the study. LAI measured by Sun-Scan canopy analysis system was calibrated with destructive measurements, showing high correlation ($n = 14$, $r^2 = 0.99$). Maximum LAI (average of all vines) was 4.2 and 4.6 m^2 m^{-2} in 2002 and 2003, respectively. The increase of ET_c along the season was explained mainly by the increase in LAI, as shown by the ET_c-LAI correlation for 2002 ($n = 50$, $r^2 = 0.74$).

EFFECTS OF PRUNING SYSTEM ON PHYSIOLOGY, LEAF AREA AND SOURCE-SINK RATION OF *VITIS VINIFERA* L. CV. VERDEJO

S. Lopez-Miranda[a], J. Yuste[a] and J.R. Lissarrague[b]

[a]*I.T.A. de Castilla y León, Ctra. Burgos km. 119, 47071 Valladolid, Spain*
[b]*E.T.S.I. Agrónomos de Madrid, Ciudad Universitaria s/n, 28080 Madrid. Spain*

Mechanization of vineyard cultural practices may necessitate the use of specific trellis/training systems. Mechanization of pruning operations generally will result in the utilization of spurs of varying lengths. A three-year study was conducted to compare short and long canes left after pruning while maintaining the same number of shoots per vine. *Vitis vinifera* L. cv. Verdejo grafted onto 110R in the Rueda Appellation of Origin (Valladolid, Spain) were used in the study. Various parameters of vine physiology, including water relations were measured, leaf area development monitored and yield and fruit quality were determined as a function of pruning type. Water relations of the vines measured at 0900 hours demonstrated no significant differences between the pruning treatments. Canopy leaf surface areas (which may give an approximation of potential canopy photosynthesis) were not significantly different between the treatments. The lack of significant differences in yield between the treatments indicates that there has been no alteration in the source-sink relationships of the vines. The similarities between the two pruning treatments in physiology activity, leaf area development and productivity and the lack of differences in must composition indicates that the mechanization of pruning may be acceptable at this location in Spain.

DESCRIPTION OF THE VARIABILITY BETWEEN *VITIS RIPARIA* VARIETIES IN TERM OF DEVELOPMENTAL AND GROWTH CHARACTERISTICS

N. Ollat, J-P. Tandonnet, M. Neveux, L. Bordenave, and S. Decroocq

Unité de recherches sur les Espèces Fruitières et la Vigne, INRA, CR de Bordeaux, BP81, F-33883 Villenave d'Ornon, France

In many premium wine grape vineyards, the rootstock is used to control vegetative growth and yield of the scion. In France, the variety *Vitis Riparia* "Gloire de Montpellier" is commonly chosen by the growers to influence these parameters. In order to provide new rootstocks inducing the same effects, a description of 20 varieties of *Vitis riparia* was undertaken. The different varieties were studied as cuttings and as a rootstock grafted with *Vitis vinifera* cv. Merlot noir. Rooting and grafting capacities were first evaluated. Budbreak and shoot growth of both cuttings and grafts were measured under greenhouse conditions. Pruning wood weight was determined under both greenhouse and vineyard conditions. A large variability existed for most characters. Rooting capacities, ranged from 1 to 11 roots per cutting. No variety was totally recalcitrant but, for two varieties, the number of rooted cuttings was very low. Grafting ability ranged from 30 to 90%. In the greenhouse, date of budbreak ranged from mid-February for the earliest varieties to mid-March for the latest. At budbreak there was a significant correlation between phenological stages registered on cuttings and those registered on grafts. On the grafts, shoot length before topping was affected by rootstock variety, however, in most cases the effect was due to only one variety which strongly inhibited growth. Grafting with Merlot induced a significant increase in pruning weights for three consecutives seasons in comparison to the cuttings. There was no relationship between the pruning weights recorded for cuttings and the ones recorded for the grafts. In the greenhouse, the rootstock effect was not significant among the grafts, although some varieties decreased pruning weights. In the vineyard, preliminary results confirmed differences among *Vitis riparia* varieties in terms of conferred vigour to the scion.

WHOLE-CANOPY VERSUS SINGLE-LEAF GAS EXCHANGE RESPONSES TO PARTIAL ROOTZONE DRYING IN POTTED CABERNET SAUVIGNON GRAPEVINES

S. Poni and F. Bernizzoni

Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Piacenza, Italy

Responses of single leaf and whole-canopy gas exchange were monitored for two weeks (29 July – 11 August) on four 5-yr-old Cabernet S. grapevines (leaf area of about 6 m^2) trained to bilateral Guyot grown with the root system split into two 40 L pots. On July 28, two vines were assigned to the treatment DRY in which water was withheld from one pot while the second was kept well watered. The two remaining vines had both pots kept well watered (WET treatment). The dry pot was re-watered on 12 August. One half of each canopy was enclosed in a flow-through whole-canopy "balloon-type" chamber system able to provide continuous recording of CO_2 and H_2O differentials across the chambers. The other half of the canopy was used for concurrent single-leaf measurements of assimilation (A), stomatal conductance (g_s), leaf transpiration (E) and leaf water potential (ψ_l) taken at 2, 5, 9, 12 days after stress and at re-watering. Volumetric soil content was monitored at a depth of 30 cm by Time Domain Reflectometry, while pre-dawn leaf water potential (ψ_{pd}) was recorded at 11 days after stress. Soil moisture approached the wilting point (≅ 9.9 % vol.) in the dry pot at day 2 of stress while ψ_{pd} in the DRY treatment (-0.25 MPa) was not statistically lower than the ψ_{pd} of WET (-1.73 MPa) at day 11 of stress. Whole-canopy net CO_2 exchange rate (NCER) was reduced about 25% in DRY over the whole stress period in that resulting similar to the A limitation detected on a single leaf basis (-28%). Diurnal NCER trends plotted for both treatments at 12 days after stress (8 August) highlighted that rates were similar till about 11 AM and, thereafter, NCER of the DRY vines started to become consistently lower than NCER rates measured on WET for the remainder of the day. Data taken the day after re-watering (12 August) showed full NCER recovery while A determined on a single leaf basis was still lower in DRY (-21%). Leaf g_s and E were reduced in DRY over the whole stress period by 23% and 18% as compared to WET, respectively, while the water use evaluated on a whole-canopy basis diminished in DRY by only 10% as compared to pre-stress levels. Intrinsic water use efficiency (A/g_s) calculated from single leaf readings over the 2-week stress period was almost identical for both treatments (≅ 40 μmol CO_2/mol H_2O).

EFFECTS OF DEFOLIATION ON TEMPERATURE AND WETNESS OF GRAPEVINE BERRIES

P. Pieri[a] and M. Fermaud[b]

[a] *ECAV, INRA, BP81, 33883 Villenave d'Ornon Cedex, France* :pieri@bordeaux.inra.fr
[b] *Santé végétale, INRA, BP81, 33883 Villenave d'Ornon Cedex, France :* fermaud@bordeaux.inra.fr

The epidemiology of *Botrytis cinerea* and others significant cluster pathogens and the maturation of grapevine berries are strongly influenced by the microclimate around the fruit. Berry temperature can affect physiological functions involved in fruit maturation and also upon pathogen growth, while berry surface wetness can affect spore germination and therefore pathogen infection. Berry and cluster microclimate can be greatly modified by changes in the training system or management techniques. The aim of this study was to quantify and analyze the response of the berry microclimate to modifications in nearby leaf area density following a defoliation operation. Temperature and berry wetness were measured with and without defoliation around the clusters in a Merlot vineyard of the Bordeaux area, fully representative of a vertically trellised training system. Berries were characterized according to cluster position (east- or west-facing), berry position within the cluster (exposed to direct sunlight illumination, internal or opposed), and defoliation treatment. Results indicated that berry temperature was strongly dependent on direct sunlight illumination pattern and duration. Temperature of exposed berries could rise 10 °C above ambient temperature, resulting in extremely elevated temperatures in west-looking and defoliated clusters during the afternoon. Berry temperature exhibited a damped variation for the non-defoliated treatment since direct sunlight illumination was weak, short-lived and erratic, according to foliage arrangement. Rainy days aside, nocturnal dew led to frequent wetness of the berries. Defoliated clusters were less protected against nocturnal long wave radiation cooling and therefore more favorable to dew formation. West-facing clusters also had longer wetness duration since they were not heated in the early morning by solar radiation.

DOWN-REGULATION OF PHOTOSYNTHETIC ACTIVITY IN OPEN FIELD VINEYARDS RELATED TO DRY CONDITIONS AND GRAPEVINE GENOTYPE

O. Silvestroni[a], S. Mattioli[a], A. Palliotti[b], A. Cartechini[b], and D. Neri[c]

[a]*Dept. of Environmental and Crop Science, Marche Polytechnic University, Italy*
[b]*Dept. of Arboriculture and Plant Protection, University of Perugia, Italy*
[c]*Dept. of Energetics, Marche Polytechnic University, Italy*

A comparative study on the adaptive responses of seven-year-old Sangiovese and Montepulciana grapevines to dry conditions (i.e. high air temperature and water deprivation) was carried out in a vineyard located on a southern-exposed hillside in central Italy (Ancona 43°40' N). The vines were spur-pruned and cordon trained. In August 2003, during the hottest portion of clear days (PAR higher than 1700 µmol photons m^{-2} s^{-1}), a pronounced down-regulation of photosystem II (PSII) activity, underlined by depression in the ratio of variable fluorescence to maximum fluorescence (*Fv/Fm*), was found on mature leaves of cv. Sangiovese vines. Under the same conditions, only a slight decrease of *Fv/Fm* was observed in mature leaves of Montepulciano and in all cases the PSII efficiency recovered the next morning. In Sangiovese leaves, the drastic reduction of *Fv/Fm* was followed by PSII damage (reversible and irreversible photoinhibition). No significant change in the photochemical efficiency was observed when the day was completely overcast (PAR of about 250-300 µmol photons m^{-2} s^{-1}) especially on young leaves. At the same time, at midday of sunny days, in comparison to cv. Montepulciano, in the leaves near the cluster of cv. Sangiovese the following other significant changes were observed:

- an increase of *Fo* yield (indicates high emission by excited chlorophyll a molecules in the antennae structure of PSII and evidenced a initial PSII damage);
- a reduction of *Fm* yield (indicates a lower photochemical potential);
- a reduction of *Fv* yield (indicates impairment of PSII activity);
- a reduction of the pool size of the electron acceptors (plastoquinone) on the reducing side of PSII (calculated as the area above the fluorescence curve between *Fo* and *Fm*);
- a reduction of the time required to obtain the maximal fluorescence (*Fm*).

The down-regulation of PSII activity and the irreversible photoinhibition phenomena (i.e. spots of necrosis up to leaf drop) will be discussed in relation to changes in net photosynthesis and characteristics involved in drought adaptation mechanisms such as stomatal sensitivity (i.e. relative water content, concentration of photosynthetic pigments, optical properties, etc.) which seem to be cultivar dependent.

A POLYGALACTURONASE INHIBITING PROTEIN FROM *VITIS VINIFERA* AFFECTS POLYGALACTURONASE ACTIVITY FROM *BOTRYTIS CINEREA* IN VIVO, IRRESPECTIVE OF SPECIFICITY

D.A. Joubert and M.A. Vivier

Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, Stellenbosch, South Africa

Polygalacturonase inhibiting proteins (PGIPs) are thought to retard fungal infection by preventing tissue maceration via an interaction with polygalacturonases (PGs) from invading fungi. This interaction also results in the production of long chain oligogalacturonides that in turn can activate several defense related pathways in the plant. This is reflected in transgenic *Nicotiana tabacum* plants overexpressing the *pgip1* gene from *Vitis vinifera* (*Vvpgip1*) that showed decreased susceptibility against *Botrytis cinerea*. Crude protein extracts from *N. benthamiana* plants overexpressing *Vvpgip1* also inhibited crude PG extracts from *B. cinerea*. We investigated whether *Vvpgip1* overexpressed in *N. benthamiana* conferred any protection to the plant against PGs from *B. cinerea*. Two PG encoding genes from *B. cinerea* (*Bcpg1* and *Bcpg2*) were overexpressed alone, or in combination with *Vvpgip1* in *N. benthamiana* leaves using *Agrobacterium* infiltration. Chlorophyll fluorescence measurements in leaves of *N. benthamiana* overexpressing *Bcpg1* and *Bcpg2* indicated a severe loss of photosynthetic ability. The effect of both BcPG1 and BcPG2 in *N. benthamiana* leaves was, however, negated by the co-expression of VvPGIP1 with both genes. Preliminary in vitro specificity analysis also indicated that VvPGIP1 does not directly interact with BcPG2. These results suggest an alternative indirect protective role for VvPGIP1 against fungal PGs.

DEHYDRATION DIFFERENTIALLY AFFECTS THE ACCUMULATION OF SPLICED AND UNSPLICED TRANSCRIPTS OF *DEHYDRIN 1* GENES IN *V. RIPARIA* AND *V. VINIFERA* TISSUES

Huogen Xiao and Annette Nassuth

Department of Botany, University of Guelph, Guelph, Ontario, Canada

Dehydrins are proteins that accumulate in vegetative tissues subjected to various dehydrating stress conditions such as cold, drought and salinity and in seeds at later stages of embryogenesis. The amount and type of dehydrin protein appears largely determined by the amount of transcript from one or more members of the dehydrin gene family present in plants. Here, we report on the expression of the dehydrin gene *Dhn1* in wild and cultivated grapevines, *V. riparia* and *V. vinifera*. At least 2 copies of *Dhn1* are present in both *V. riparia* and *V. vinifera*, as determined by Southern blot analysis and confirmed by iPCR and sequencing of the obtained fragments. One copy in *V. vinifera* has a small 18 nt deletion compared to the other gene. Analysis by RT-PCR and sequencing of the amplified fragments showed that expression of the *Dhn1* genes could produce a spliced transcript, *Dhn1S*, and an unspliced transcript, *Dhn1U*, which potentially drive the synthesis of two different types of dehydrins with possibly different functions. Only low amounts of *Dhn1S* were detected in *V. riparia* leaves under control conditions. However, exposure to cold, drought or ABA induced a much higher accumulation of *Dhn1S*, and, after a longer cold or drought period, also of *Dhn1U*. Cold treatment of *V. vinifera* leaves gave a similar result. *V. riparia* seeds and cold treated buds also contained *Dhn1S* and *Dhn1U* transcripts, at all times sampled. In contrast, *V. vinifera* buds contained two spliced transcripts and, only after a longer cold treatment, one unspliced transcript. The possible relation of these results with the freezing tolerance of *V. riparia* and the freezing susceptibility of *V. vinifera* will be discussed.

FLORAL MERISTEM IDENTITY GENES IN GRAPEVINE

M. Calonje[a], P. Cubas[b], J.M. Martínez-Zapater[b,c] and **M.J. Carmona**[a]

[a]*Departamento de Biotecnología, Escuela Técnica Superior Ingenieros Agrónomos, Universidad Politécnica de Madrid, Avenida Complutense s/n, 28040 Madrid, Spain*
[b]*Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Campus de la Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain*
[c]*Departamento de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Carretera de La Coruña km 7, 28040 Madrid, Spain*

The flowering process in grapevine (*Vitis vinifera*) takes place in buds and extends for two consecutive growing seasons. To understand the genetic and molecular mechanisms underlying this process, we have characterized bud development, cloned the grapevine orthologs of *FLORICAULA/LEAFY* (*FLO/LFY*), *APETALA1* (*AP1*) and *FRUITFUL* (*FUL*), called *VFL*, *VAP1* and *VFUL* respectively, and analysed their expression pattern during vegetative and reproductive development. Flowering induction takes place during the first season. Upon induction, the shoot apical meristem begins to produce lateral meristems or *uncommitted primordia* that will give rise to either inflorescences or tendrils. During the second season, after a winter dormancy period, buds reactivate and inflorescence meristems give rise to flower meristems. *VFL*, *VAP1* and *VFUL* are expressed in the *uncommitted primordia* and throughout inflorescence development but, further, their expression diverged during flower development. These expression patterns are consistent with the previously described roles for these genes in reproductive development. However, the lack of expression of *VAP1* in sepal primordia during flower organogenesis, does not support its role as an A-function gene in grapevine. Additionally, these genes are also expressed in other meristematic regions and vegetative organs. These patterns of expression have important implication on our understanding of flower induction and initiation in grapevine and on the functional role of these genes in plant development.

DEVELOPMENT OF DUAL PD-DIAGNOSIS SYSTEM BASED ON HOST GENE EXPRESSION USING MULTIPLEX REAL TIME RT-PCR

Hongkyu Choi[a], Francisco Goes da Silva[a], Hyunju Lim[a], Alberto Iandolino[b], Jongmin Baek[c], Anna Leslie[c], Jane Xu[a] and Douglas R. Cook[a]

[a]*Department of Plant Pathology, University of California, Davis One Shields Avenue, Davis, CA 95616*
[b]*Department of Viticulture and Enology, University of California, Davis One Shields Avenue, Davis, CA 95616*
[c]*CAES-Genomics Facility, University of California, Davis One Shields Avenue, Davis, CA 95616*

The reliability of pathogen detection in diseased plants can be limited by sampling efficiency. In the case of Pierce's Disease (PD) of grapes, vines in the early stage of infection are particularly troublesome because such plants are asymptomatic and the pathogen often infects sectors rather than uniformly throughout the vine. These facts conspire to increase the rate of false negative diagnosis, allowing infected individuals to remain in the field and provide inoculum for neighboring, healthy individuals. An ideal diagnostic method would be sensitive enough to detect disease in asymptomatic plants, and able to diagnose disease even when non-infected portions of diseased plants are assayed. With these criteria in mind, we have been working to develop a diagnostic method based on host gene expression. Ideally such genes would be (1) induced early and specifically in response to pathogen infection, (2) expressed in both asymptomatic and symptomatic vines, and (3) induced systemically in response to localized infection. The reliability of such an assay would be increased by monitoring the co-expression of *Xylella* genes. Thus, the ultimate goal of this research is to develop a dual PD-diagnosis system that can detect plant marker gene expression as well as the presence of bacteria using multiplex real time RT-PCR. Based on expressed sequence tag (EST) profiling of infected and healthy grape vines, we have identified thirty-five putatively *Xylella*-induced transcripts. Twenty of these genes were selected for further analysis using conventional reverse transcription (RT) PCR and real time RT-PCR. Four of these genes were confirmed to be strongly up-regulated in *Xylella*-infected tissues, with at least three genes confirmed to be expressed during the early, asymptomatic phase of the disease. We anticipate that such expressed genes will contribute to our goal of developing marker genes for early PD-diagnosis.

IRRIGATION SCHEDULING OF GRAPEVINES USING MIDDAY STEM WATER POTENTIAL, WEATHER DATA AND SOIL MOISTURE MONITORING

S. Fuentes[a], R. Mora[b] and S. Ortega–Farias[b]

[a]*Centre for Horticulture and Plant Sciences (CHAPS), University of Western Sydney, Australia, E-mail: s.fuentes@uws.edu.au*
[b]*Research and Extension Centre for Irrigation and Agroclimatology (CITRA), University of Talca, Chile*

Two methods to schedule irrigations were compared in an irrigated trellising trial using *Vitis vinifera* L. (cvs. Shiraz, Merlot and Cabernet Sauvignon), located at Isla de Maipo, Chile (33° 46' 16" S; 70° 55' 34" W) in the 2002-03 growing season. The first methodology (M1) consisted of monitoring plant water status using midday stem water potential with a pressure chamber (Ψ_{stem}), soil moisture using Time Domain Reflectometry (TDR, Trase Systems) and evapotranspiration using a weather station (Davis Advantage Pro). The second methodology (M2) used only soil moisture and climatic data to obtain irrigation scheduling thresholds, for the same cultivars. Using M1 it was possible to finish the season with non-stressed vines (Ψ_{stem} maintained above –1.0 MPa) without water supplied by irrigation for Shiraz and Merlot. Also, increments in grape quality (anthocyanins content) were found for Cabernet Sauvignon under three Ψ_{stem} irrigation treatments (T1 maintained between -0.8 and –1.0 MPa; T2 between -1.0 and –1.2 MPa and T3 between -1.2 and –1.4 MPa). Yields of M1 and M2 were not significantly different for any of the cultivars. The same was found for total water application (TWA) for Shiraz and Merlot, but there were significant differences in TWA for the three irrigation treatments based on M1 using Cabernet Sauvignon. In this case, TWA (in ML/ha) were: T1 = 2.7; T2 = 1.6 and T3 = 0.22, with yields (Tons/ha), of 10.2, 8.1 and 8.9, respectively for M1, compared to 3.7 ML/ha and yields of 9.5 Tons/ha for M2. This study demonstrates the importance of including physiological parameters in grapevine irrigation scheduling and that methodologies based only on climatic and soil moisture data can mislead the real plant water status generating uncontrolled over and under irrigated vines with detrimental consequences in grape yield and quality.

INCLUSION OF PHYSIOLOGICAL PARAMETERS IN GRAPEVINE IRRIGATION SCHEDULING UNDER PARTIAL ROOT-ZONE DRYING

S. Fuentes[a], G. Kelley[a], R. Mora[b], G. Rogers[c,d], and J. Conroy[a]

[a]*Centre for Horticulture and Plant Sciences (CHAPS), University of Western Sydney, Australia. E-mail: s.fuentes@uws.edu.au*
[b]*Research and Extension Centre for Irrigation and Agroclimatology (CITRA), University of Talca, Chile*
[c]*AHR Crop Science, University of Sydney, NSW 2006, Australia*
[d]*Faculty of Agriculture, Food and Natural Resources, University of Sydney NSW, Australia*

Current methodologies for scheduling irrigation in vineyards are based on soil moisture and climatic parameters and cannot possibly detect physiological effects of temporal and spatial soil moisture distribution imposed by new irrigation methodologies, such as partial rootzone drying (PRD). The effect of PRD irrigation, compared with fully irrigated controls (FI), half irrigated controls (HI), and sub-surface (SI) irrigation on stem water potential (Ψ_{stem}), transpiration rates and soil wetting patterns (SWP), were evaluated on field-grown grapevines (*Vitis vinifera* L. cv. Shiraz) at Richmond NSW, Australia (University of Western Sydney) at the beginning of the 2003-04 growing season. The vines were irrigated when Ψ_{stem} reached values of –1.0 MPa to avoid stress. There was no significant difference in Ψ_{stem} of vines under PRD compared with controls. Transpiration rates, measured with heat-pulse sap flow probes, were lower in vines under PRD and HI controls due to a reduction in stomatal conductance. SWP were visualised in real time using Wetting Patterns Analyser (WPA©) software in all treatments and correlated with plant water status (Ψ_{stem}).

CHEMICAL COMPOSITION, C/N RATIO AND CONSTRUCTION COSTS OF GRAPEVINE TISSUES DURING ONTOGENY

Ph. Vivin, M. Castelan-Estrada, A. Gourieroux and J.P. Gaudillère

Ecophysiologie et Agronomie Viticole, UMR Oenologie & Ampélologie, INRA Bordeaux-Aquitaine, BP 81, Villenave d'Ornon, France, Vivin @bordeaux.inra.fr

In order to quantify ontogenetic changes in construction costs, the chemical compositions of leaves, stems, fruits, fine roots and trunk were determined several times during a vine's growth cycle. Tissue construction costs were estimated using either (i) an approach based on the quantification of the amount of glucose required for the synthesis of the major chemical components of the plant organs by the most probable metabolic pathways, or (ii) a simpler technique in which costs values were derived from tissue ash, carbon and nitrogen concentrations.

CHARACTERIZATION OF AN OUTWARD RECTIFYING POTASSIUM CHANNEL FROM *VITIS VINIFERA* L.

R. Pratelli[a], E. Hosy[a], B. Lacombe[a], C. Romieu[b], A. Ageorges[b], J.B. Thibaud[a], L. Torregrosa[c] and H. Sentenac[a]

[a]*Biochimie et Physiologie Moléculaire des Plantes, UMR 5004 Agro.M-CNRS-INRA-UM2,1 place Viala, 34060 Montpellier cedex 1, France.*
[b]*Sciences pour l'Œnologie, UMR 1083 Agro.M-INRA-UM1, 2 place Viala, 34060 Montpellier cedex 1, France.*
[c]*Biologie du Développement des Plantes Pérennes Cultivées, UMR 1098 Agro.M-CIRAD-INRA-IRD-UM2, 2 place Viala, 34060 Montpellier cedex 1, France.*

(Eco)physiological approaches have clearly pointed out the importance of K+ for grapevine development and for wine quality. At the whole plant level, this ion is essential for growth, photosynthesis and stomatal movements. As the major inorganic ion of the cells, K+ is involved in the electrical neutralization of organic acids in the grape berry. However, its excessive accumulation in berries results in wines with poor gustative and storage qualities (low acidity and tartaric/malic-acid ratio). We analyzed molecular determinants of K+ transport in grapevine, focusing on Shaker-like K+ channels. Here, we report on SOR, an outward rectifying channel similar to *Arabidopsis* SKOR and GORK, which is supposed to be involved in stomata aperture and in K+ loading into the berry. In an attempt to confirm these putative roles, we set up a reverse genetic approach, consisting in the transformation of vines with a dominant negative mutant of SOR channel. The first phenotypic analyses are presented.

CROP LOAD EFFECT ON SANGIOVESE GRAPEVINE

Giovan Battista Mattii[a] and Francesco Ferrini[b]

[a]*Dipartimento di Ortoflorofrutticoltura, University of Florenc, Italy* gbmattii@unifi.it
corresponding Author
[b]*Dipartimento Produzioni Vegetali, University of Milan, Italy* francesco.ferrini@unimi.it

A three year research project to evaluate the effect of crop load on fruit qualitative and quantitative production as well as vegetative performance was conducted. Research was carried out in 2000, 2001 and 2002 in a 10-year-old Sangiovese/420 A vineyard. The vines were planted to a 3x1 m spacing and vertical trained as a single curtain and spur pruned. Ten crop load treatments were imposed by cluster thinning at veraison. Cluster number/vine were 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 20. Yield was strictly related to cluster number left after thinning, allowing to easily manage production quantity by regulating crop load at veraison time. Berry and cluster weight were not correlated with cluster thinning. Crop load played an important role on grape quality characteristics. Sugar content in the must was inversely correlated to cluster number as well as to yield in all the years of study, and the differences among crop load treatments were more marked during the first period of the ripening process. Good correlations were also found between crop load and both titratable acidity and must pH as well as anthocyanins content. Cluster thinning didn't affect vegetative growth or leaf gas exchange.

PROTEOMIC ANALYSIS OF GRAPEVINE RESPONSES TO WATER DEFICIT AND SALT STRESS

Delphine Vincent, Ali Ergul, Marlene C. Bolhman, Elizabeth Tattersall, Richard Tillett, Monirath Sali, Rebekah Woosley, Dave Quilici, David Schooley, John Cushman and Grant R. Cramer

Department of Biochemistry, University of Nevada, Reno, NV 89503

A global project on *Vitis vinifera* integrating all gene expression levels has been undertaken to study plant adjustment to various abiotic stresses in relation to plant tolerance and wine quality. The objectives are to develop improved strategies for enhancing stress tolerance mechanisms in grapevine but also provide important insights into the molecular basis of stress tolerance and underlying mechanisms of how stress affects wine quality. Investigating all gene expression levels (transcripts, proteins and metabolites) will allow us to correlate them and confirm the involvement of particular genes in the grape response to drought, cold and salt stresses. Here we present a proteomic analysis of a long-term stress experiment on the worldwide grown cultivars, Chardonnay and Cabernet Sauvignon, subjected to water deficit or salt stress. Growing shoots were collected at successive developmental stages after stress application (day 0, 6, 12 and 18) in order to observe the stress responses over time. Some physiological measurements such as water potential, shoot and leaf growth were taken. Proteins were extracted with trichloroacetic acid/acetone and separated by Two-Dimensional-Polyacrylamide Gel Electrophoresis (2D-PAGE). Protein amounts were analyzed using PDQuest software and proteins were identified using Matrix Assisted Laser Desorption Ionization Time of Flight/Time of Flight (MALDI-TOF/TOF). Data analysis indicates that the variation of protein amount is mainly associated with developmental stages and that salt and water stresses affect grape physiology as well as protein expression over time. Most of the excised spots (186) have led to a clear identification of the proteins. Some identified proteins known to respond to stress (SOD, APX) are not necessarily affected by abiotic stress in our conditions.

CARBOHYDRATE CONTENT IN INFLORESCENCES OF GRAPEVINE (*VITIS VINIFERA* L.) AND SEXUAL STRUCTURE DEVELOPMENT IN RELATION WITH FLOWER AND FRUIT DROP

Gaël Lebon[a], Christian Magné[a], Olivier Brun[b] and Christophe Clément[a]

[a]*Laboratoire de Stress, Défenses et Reproduction des Plantes, URVVC UPRES EA 2069, Université de Reims Champagne Ardenne, UFR Sciences, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France*
[b]*Mumm-Perrier-Jouet Vignobles et Recherches, Avenue de Champagne, 51206 Epernay, France*

In order to better understand abnormal flower and berry abortion in grapevine (*Vitis vinifera* L.), we examined 1) the chronology of male and female organogenesis, and 2) the variations of carbohydrate content of the inflorescence during flower development. The cultivars studied were the flower abscission sensitive Gewurztraminer (GW) and the non-sensitive Pinot noir (PN) grown in vineyard. Of the two cultivars tested, the development of reproductive organs was more precocious for PN. During pollen development, meiosis occurred between stages 12 (Eichhorn and Lorenz classification) and 15 in the PN but 2-6 days later, between stages 15 and 15+2 days (d), in GW. The microspores underwent mitosis between stages 15+8 d and 17 in PN and 10 days later between stages 17 and 21 in GW. Pollen grains were mature at flowering in both cultivars. Meiosis in the ovules took place between the stages 15+2 d and 15+8 d for PN and 2 days later between stages 15+8 d and 17, for GW. In both cultivars, the embryo sac had fully developed at stage 21. Carbohydrate contents were significantly different between the two cultivars during flower development. The differences occurred at stage 15+8 d; starch and sucrose contents were higher in the inflorescences of GW when compared to PN while glucose and fructose were higher in PN. This stage coincides to microspore and embryo sac mother cell development in PN, whereas it corresponds to microspore and megaspore mother cell development in GW. These developmental stages are crucial steps in obtaining functional sexual organs. The data indicate that the amount and type of carbohydrates in the inflorescences at various developmental stages of reproductive development may be involved in the differential sensitivity to flower abscission of these two cultivars.

CHILEAN EFFORT FOR IMPROVING FRUIT QUALITY IN GRAPEVINE: A GENOMIC APPROACH TO UNDERSTAND SEED FORMATION, FRUIT RIPENING AND RESPONSE TO PATHOGEN

Hugo Peña-Cortés[a], Jorge Valdés[a], Manuel Pinto[b], Alejandro Riquelme[b], Tomas Fitchet[b], Patricio Hinirchsen[c], Humberto Prieto[c], Marlene Rosales[c], Danilo González[d], Matilde Jashes[d], Simón Ruíz[e], Enrique González[e].

[a] *Universidad Técnica Federico Santa María,. Valparaíso*
[b] *Universidad de Chile, Santiago*
[c] *Instituto de Investigaciones Agropecuarias, Santiago*
[d] *Universidad de Santiago de Chile, Santiago*
[e] *Universidad de Talca, Talca.*

Improving fruit quality is a priority for the Chilean grape industry. A national effort has begun using the genomic approach to study problems related to seed formation, fruit ripening and the vine's response to *Botrytis cinerea* infection. Seedless cultivars such as Thompson Seedless have embryo abortion at an early stage of berry growth which affects seed formation and subsequently the size of the berry. To obtain berries of commercial size clusters are treated with gibberellic acid (GA_3). The genes and/or biological processes controlling embryo abortion and fruit size, as well as the response of berries to GA_3 applications in this cultivar are unknown. On the other hand, wine grape cultivars such as Carménère also have problems related to seed formation and berry size. Clusters containing a high proportion of small seedless berries can negatively affect wine quality. The causes for this are also poorly understood. Finally, berries of both Thompson Seedless and Carménère are affected by *Botrytis cinerea*. It appears that Carménère is more tolerant to the infection than Thompson Seedless. The genes and/or biological processes regulating these responses are unknown. We have begun a functional genomic approach towards defining the changes that occur during fruit development in both cultivars. We are in the process of sequencing approximately 100.000 ESTs from flowers and fruits collected at different stages and either sprayed or not sprayed with GA_3. The clones and sequence information generated will be used to analyze the global gene expression using macroarray analysis.

Funding provided by Iniciativa Genoma Chile FONDEF G02P1002

THE SIGNIFICANCE OF PHOTOINHIBITION AND SUNFLECK PHOTOSYNTHESIS OF GRAPEVINES USING THE OVERHEAD TRELLIS SYSTEM

M. Pinto, M. Berti, C. Pacheco and V. García de Cortazar

Universidad de Chile, Facultad de Ciencias Agronómicas, Casilla 1004, Santiago, Chile

The overhead trellis system is commonly used for table grape production in Chile. This study defined three illumination zones within this trellis system: 1.) a zone where leaves are illuminated for most of day to very high intensities, greater than 2000 $\mu mol\ m^{-2}\ s^{-1}$, 2.) a zone where leaves receive light predominantly as sunflecks and 3.) a zone where leaves predominantly receive diffuse radiation, intensities less than 100 $\mu mol\ m^{-2}\ s^{-1}$. The daily courses of current photochemical capacity and CO_2 assimilation rates revealed that photoinhibition was a common phenomenon in leaves located in the first illumination zone. However, no permanent photo-damage was observed. Recovery in these leaves took place shortly after midday, continuing throughout the night and reaching normal predawn values the next morning. This daily cycle with periods of photoinhibition and recovery, was also observed in leaves located within the sunfleck zone. However, in this case fluctuations were much less pronounced. Current photochemical capacity was always high (0.8) throughout the day in leaves located in the third zone. As the contribution of the first illumination zone to total vine photosynthesis was low (10 %), it was concluded that photoinhibition in these leaves had a rather low impact on the total carbon economy of the plant. In this system most of the carbon for the berry growth would come from leaves located in the sunfleck zone. This stresses the importance of knowing the photosynthetic behavior of grapevine leaves under these conditions. A description of the sunfleck regime in the different illumination zones throughout the day was completed. A close correlation (r^2= 0.83) between the epoxidation state (EPS) and the current photochemical capacity throughout the day was found.

This work was supported by Fondecyt, Chile Grant Nº 1000839

RESEARCH TO PRACTICE® WINEGRAPE QUALITY MANAGEMENT - A SPECIALISED VITICULTURAL TRAINING PROGRAM IN AUSTRALIA

Erika Winter[a], Robert Sward[a], Karen Green[a], and Peter Mansfield[b]

[a]*Department of Primary Industries Victoria, Private Bag 15, Ferntree Gully Delivery Centre, VIC 3165, Australia*
[b]*Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, SA, 5064, Australia*

The aim of Research to Practice training programs in Australia is the adoption of research results through nationally consistent training, based on adult learning principles. Training in viticulture is delivered over 2 days in an interactive workshop, encompassing a take home manual. Participants are assisted to develop a commitment for a practice change, which is reviewed and refined at a third day session after harvest. The workshops on Winegrape Quality Management encompassed presentations and exercises to teach growers how to avoid the occurrence of negative specifications, assess and promote vine balance, react to the phenology of berry development, influence sugar, acidity and pH development, understand the origin of secondary metabolites, assess and influence colour development and how to influence aroma development. Evaluation of the feedback sheets from 698 participants trained in 36 workshops showed patterns of differing regional interests in the topics of the workshop. Forty-seven percent had a strong preference for learning about Vine balance, 22% found all topics interesting, Aroma management was the favourite topic in 17%, mainly cool climate locations, and outstanding preference for the Colour topic was seen in groups growing grapes in warm climates. Approximately 70% of participants returned for a 3rd Day session. They reported that 58% of their knowledge that led to practice changes was obtained in the training course. Testimonials provided showed cost savings and higher income for better grapes as a result of changed practice. Participants were more motivated and networking improved due to the workshops. Also a better understanding of grape physiology led to more sustainable practices.

ISOLATION AND PRELIMINARY CHARACTERIZATION OF VVMYB3, A NOVEL MYB TRANSCRIPTION FACTOR IN GRAPE

Carra A., Schubert A. and Martin C.

Dipartimento di Colture arboree, Università di Torino, Italy
Department of Cell and Developmental Biology, John Innes Centre, Norwich, UK

MYB transcription factors constitute the largest known class of regulatory proteins in plants. They are involved in a wide range of biological processes which include phenylpropanoid metabolism. Knowledge of MYB transcription factors is based largely on data obtained in model species, while little is know about the presence and the functions of these genes in fruit plants, despite their important economic interest. To date, four MYB transcription factors have been isolated in grape. To one of these MYB genes, *VlMYBA* from *Vitis labruscana*, it has been assigned as a putative function the transcriptional activation of *UFGT*, the gene responsible for the last step in anthocyanin biosynthesis. We isolated VvMYB3, a novel MYB transcription factor from grape (*Vitis vinifera* L.) by 3' RACE, taking advantage from the highly conserved sequence of the N-terminal binding domain. By sequence analysis VvMYB3 has been placed within the subgroup 4 of MYB transcription factors, that comprises well known regulators of the early steps of phenylpropanoid metabolism like AtMYB4 from *Arabidopsis* and AmMYB308 from *Antirrhinum*. Furthermore, the members of the subgroup 4, in contrast to the majority of the MYB proteins, act as transcriptional repressors rather than as activators. We therefore hypothesise that VvMYB3 has a similar function. In order to test this hypothesis, we expressed the gene in tobacco in the sense direction. Most sense direct transformants showed no expression of the gene, possibly due to silencing. Selected first generation transformants strongly expressed the gene: these plants exhibited a dwarf phenotype with chlorotic leaves carrying necrotic lesions. In these transformants the expression of C4H and 4CL was assessed. The study of the pattern of expression of VvMYB4 in grapevine and a further characterization of tobacco sense transformants are underway.

DIFFERENTIAL SCREENING TO ISOLATE GRAPE GENES RELATED WITH VIRAL INFECTIONS

C. Espinoza, C. Medina, and P. Arce-Johnson

Laboratorio de Bioquímica, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. Santiago, Chile. e-mail: parce@genes.bio.puc.cl

One of the main problems related with grape cultures are the infections caused by pathogens like bacteria, fungi and viruses. Viral infections have a high incidence, are also difficult to control and affect grapes quality, causing important economic losses. Until now, more than forty viral strains have been described that can infect grape cultures, generating chronic diseases without any resistance reaction associated. In spite of the efforts made in the discover of grape genes involved in abiotic stress, less work have been done in the identification of those genes that change their expression level in response to viral infections. In order to understand how grapevine plants face this problem, we used plants of V. vinifera cv. Carménère infected with Grapevine Leafroll Virus and healthy plants of the same variety to construct two cDNAs libraries using a subtractive hybridization approach, which allow us to characterize genes which are related to viral disease. A total of 455 and 350 clones from forward and reverse library, respectively, have been amplified by PCR. In both libraries, about 60-70% of the analyzed clones have inserts amplified as single bands in a range size between 400 and 1000 bp. In order to have a first approach to identify differentially expressed clones we performed a differential screening of both libraries using a macroarray approach. For this, 346 forward clones and 275 reverse clones have been printed onto nylon membranes and hybridized with cDNA probes made from healthy and infected tissues. After this preliminary screening we have selected 54 forward clones and 27 reverse clones that could be related with viral response. Results from clone sequencing will be discussed.

Acknowledgements: This work is supported by FONDEF grant G02S1001.

SIMULATIONS OF BUD BREAK DATES

I. García de Cortázar [1*], N. Brisson [1], B. Seguin [2], J.P. Gaudillere [3], B. Baculat [2]

(1) Unite Climat Sol Environement, INRA, Site Agroparc, domaine St-Paul, F-84914, Avignon cedex 9, France
(2) Unite Agroclim, INRA, Site Agroparc, domaine St-Paul, F-84914, Avignon cedex 9, France
(3) UMR Oenologie & Ampélologie, INRA, Domaine de la Grande Ferrade - BP 81, F-33883, Villenave d'Ornon cedex, France
**Corresponding author email: igarcia@avignon.inra.fr*

Some effects of the climatic change are already observable in certain aspects of grapevine behaviour, among which phenology is the most significant (both advanced flowering and vintage). The bud break stage is very important because it determines the beginning of the cycle and at the same time it is very variable among locations and varieties. The objective of this work is to develop a reliable bud break model, for which we have used the data base PHENOCLIM®. We have used a selection of 9 types of varieties (Cabernet Franc, Cabernet Sauvignon, Chardonnay, Grenache, Merlot, Pinot Noir, Riesling, Syrah, Ugni Blanc) in five different sites. First, we have tested the already existing bud breaks models: the number of days for bud break; the sum of degree days and the sum of "Actions day" calculated using Riou's model (1994). A major disadvantage of these models is that they do not take into account the dormancy period. They compute the bud break since January first. The predictions of these models display many discrepancies with the observed dates, and these results depend so much on the varieties and the sites making a generalization quite impossible. So, in the second step, we have build a new bud break model BRIN. Its innovation is to take into account the dormancy period in the calculation of the bud break. It uses the Bidabé's phenologic model of "Cold actions" (1965), to compute the end of "real" dormancy and the Richardson's GDH model (Growing Degrees Hours) for the computation of the bud break from this time on. After model calibration, validation was performed using an independent data set. The model allows the calculation of bud break over all locations for known varieties.

FUNCTIONAL GENOMICS PROJECT IN GRAPEVINE: GENE EXPRESSION IN RESPONSE TO VIRAL INFECTIONS

P. Arce-Johnson

Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile. e-mail: parce@genes.bio.puc.cl

Grapes are cultured in Chile for fruit exportation and wine industry, constituting the major area of national agriculture. The grapevine genomics project pretends to extend in Chile the use of genomics technologies to improve the knowledge of this species with emphasis in the response to viral infection. In the long time we pretend to improve the control and prevention of viral diseases. We present our advances in the different work areas of the project. Grape genes that respond to viral infection have been detected using substactive hybridization libraries and AFLP-TP technology. Genes that appears up and down regulated as a consequence of viral infection after a preliminary screening are shown. On the other hand, several Chilean isolates of important grapevine viruses are being sequenced. Results of the Grapevine Fan Leaf Virus and Grape Vine Leaf Roll Virus sequences are shown. Some viral proteins are being expressed and they will be used to develop specific diagnose kits. This project also includes the implementation of a genomic database of grapes EST sequences upgraded with re-annotation of genes according to Gene Ontology Consortium. Advances in genetic transformation of *N. benthamiana* and grapes tissues by use of *Agrobacterium*, in order to obtain viral resistant plants are presented.

Acknowledgements: Fondef project G02S1001.

COMPARATIVE MAPPING OF QTLS IN GRAPE

Laura Costantini, Alberto Madini, Juri Battilana, M. Stella Grando

Istituto Agrario di San Michele all'Adige – Via Mach, 1 38010 Trento ITALY

Over the past few years, a lot of efforts has gone into establishing the first linkage maps for grape. Most of the mapping populations have been interspecific hybrids often chosen because the parents are sources of disease resistance (Lodhi et al. 1995, Dalbò et al. 2000, Callahan et al. 2002, Grando et al. 2003, Fischer et al. 2004). Currently, a number of *vinifera* x *vinifera* crosses are being employed for table grape quality trait mapping experiments (Doligez et al. 2002, Zavala et al. 2002, Costantini et al. 2003, Gieco et al. 2003) and the reference linkage map adopted by the International Grape Genome Program (IGGP, http://www.vitaceae.org) is based on a Riesling x Cabernet Sauvignon cross (Riaz et al. 2004). The number of molecular marker segregation data sets available for grape should allow soon a consensus map to be generated on which fine scale genetic maps including functional marker types could be integrated. Moreover, this valuable tool will enable the grape community to extricate and utilize information from distantly related taxa as genetic mapping reveals that a remarkable diversity of form among extant plant taxa is accompanied by many fundamental similarities (Paterson, 1997). We have in progress several mapping experiments aiming to dissect the genetic basis of quality traits of the berry and resistance to fungal pathogens. Here we present the first comparison of marker order and QTL position results from two independent mapping projects based on different *Vitis* genetic backgrounds.

RESPONSE OF FLAME SEEDLESS GRAPEVINES TO SOME NITRIFICATION INHIBITORS

Samy EL-Shazly

Faculty of Agriculture, Alexandria University, Egypt

A field study was conducted to determine the effects of several nitrification inhibitors on Flame Seedless grapevines grown in a sandy loam soil. The nitrification inhibitors (NI) used in the present study were nitrapyrin at rates of 2.5, 5 and 10 mg/L and 8-Hydroxyquinolin (8-HQ) and carbon disulphide (CS2) at rates of 10, 20 and 40 mg/L. For two seasons, nitrapyrin at all tested rates and CS2 at 20 and 40 mg/L significantly increased all vegetative growth parameters (shoot length, leaf area and pruning weight) as compared with the control. Furthermore, 8-HQ at 20 and 40 mg/L markedly increased shoot length and slightly increased leaf area and pruning weight. The number of clusters/vine and yield (kg/vine) were not significantly affected by the NI the first season. Nitrapyrin at 5 and 10 mg/L and CS2 at 40 mg/L significantly increased the number of clusters/vine and yield the second season. Nitrapyrin and CS2 at medium and high rates markedly increased cluster weight and weight and juice volume of the berries, total soluble solids (TSS) and vitamin C content both seasons. The 8-HQ treatment at 20 mg/L significantly increased cluster weight, berry weight and juice volume both seasons and markedly increased TSS and vitamin C content the second year. None of the NI treatments significantly affected juice acidity. Leaf N, Fe, Mn, Zn and Cu contents were significantly increased as a result of NI applications. Leaf P, K and Ca contents decreased, especially at high NI rates. The addition of NIs significantly increased soil NH4+-N but decreased soil NO3--N and soluble N both seasons. The effect of NI on nitrification rate and ammonium recovery ratio will be discussed.

EXPRESSION ANALYSIS OF SUGAR TRANSPORTERS AND INVERTASES DURING GRAPE BERRY RIPENING

Matthew A. Hayes, Christopher Davies and Ian B. Dry

CSIRO Plant Industry, Horticulture Unit, PO Box 350, Glen Osmond, SA 5064, Australia; University of Adelaide, School of Horticulture & Wine, Glen Osmond, SA 5064 and Cooperative Research Centre for Viticulture, PO Box 145, Glen Osmond, SA 5064, Australia

The ripening of grape berries is marked by massive accumulation of hexoses in the vacuoles of berry pericarp cells. To investigate the possible role of sugar transporters and invertases in this process, a real time, reverse transcription study of the expression of these genes during grape berry ripening was undertaken. Three previously unreported hexose transporters, VvHT2, VvHT3, VvHT4, and a cell wall invertase, VvCWINV1, were cloned from grapevine using degenerate PCR. Full length cDNAs were obtained for each transcript using RACE PCR, and the functionality of VvHT3, VvHT4 and the previously reported VvHT1 was confirmed in the yeast expression system. Using real-time reverse transcription PCR, the expression of VvHT2, VvHT3, VvHT4 and VvCWINV1 were analysed together with VvHT1, sucrose transportersVvSUC12 and VvSUC11 and three ESTs with homology to cytoplasmic invertases. Total RNA was extracted from various tissues of *Vitis vinifera* cultivars Cabernet Sauvignon and Shiraz, including roots, shoots, leaves, and berries approximately two, six, ten and fourteen weeks post flowering. The six sugar transporters investigated showed different expression profiles, with VvHT2, VvSUC11 and VvSUC12 expression increasing during berry development. Of the invertases investigated, VvCWINV1 was expressed strongly at late stages of berry ripening and one EST with homology to cytoplasmic invertases increased in expression during berry ripening. These studies allow the development of a model describing the involvement of sugar transporters and invertases in the apoplastic route of sugar loading into grape berries.

THE EFFECTS OF DRIP IRRIGATION ON VINE PHISIOLOGY

Aurora Ranca, Ghita Marin, Cornelia Bian and Diana Braduceanu

Research Station for Viticulture and Enology Murfatlar, Basarabi, 8764, Constanta Romania, e-mail: scv_murf@1a.ro

Drip irrigation was installed in a vineyard at Murfatlar for the 2002 and 2003 growing seasons. The system consisted of pressure compensated in-line emitters. The cultivar used was Columna (a cross of Pinot gris and Grasa de Cotnari –an authchthon cultivar, created by scientists at Murfatlar, and used for making white quality, white dry and half-dry wines). The training system was a Guyot with half-trunk and planting density was 2 x 2.5 m. The rootstock was the *Berlandieri* x *Riparia* cross (SO4). The amount and distribution of rainfall at Murfatlar was irregular across both years. The purpose of this research was to investigate the relationships between soil water status and physiological processes and harvest quality and quantity. Irrigation was carried out from flowering (anthesis) until three weeks prior to harvest. The soil water content was determined by the gravimetric method. Photosynthesis and stomatal conductance was determined using the LiCor system. The measurements were made at the following phenological events: flowering, veraison and close to harvest. Comparisons were made between irrigated and non-irrigated Columna vines. The measured physiological processes between treatments were similar as the vines developed early on, after that photosynthesis rate and stomatal conductance decreased until the end of August followed by a slow increase into September. Yield of the drip-irrigated plot was 20% greater than that from the non-irrigated plot. Grape quality at harvest was assessed by measuring sugars and acids and glycosides determined with an inductile plasma spectrophotometer.

Senior Author Index