TECHNICAL

abstracts

AMERICAN

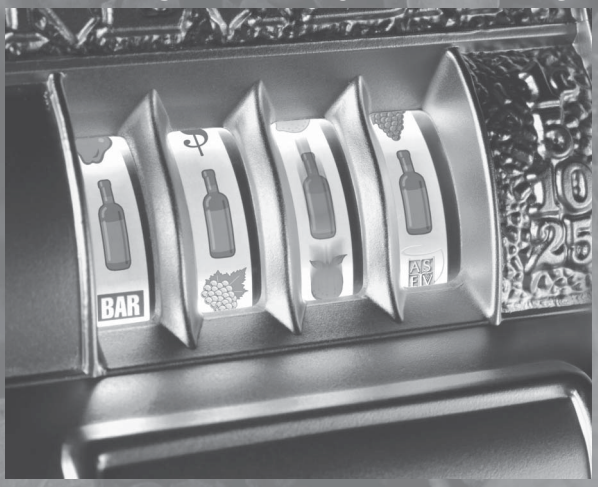
SOCIETY FOR

ENOLOGY AND

VITICULTURE

asev

54TH ANNUAL MEETING



JUNE 18–20, 2003

RENO HILTON

RENO, NEVADA

TECHNICAL

abstracts

ORAL AND

POSTER

PRESENTATIONS

AMERICAN

SOCIETY FOR

ENOLOGY AND

VITICULTURE

54TH ANNUAL MEETING

JUNE 18–20, 2003

RENO HILTON

RENO, NEVADA

2003 Technical Program Committee

Signe Zoller, Meridian Vineyards	Program Committee Chair
Andrew Waterhouse, UC Davis	General Sessions Co-Chair
Andrew Walker, UC Davis	General Sessions Co-Chair
Patricia Howe, UC Davis	Poster Sessions Chair

Session Chairs

Andrew Waterhouse, UC Davis	Color and Phenolics
Grant Cramer, University of Nevada, Reno	Viticultural Genetics
David Block, UC Davis	Effects of Viticultural/Enological Practices on Wine Composition/ Quality
Linda Bisson, UC Davis	Yeast Fermentation Biology
Andrew Walker, UC Davis	Progress on Viticultural Diseases
Sara Spayd, Washington State University	Wine Chemistry/Microbiology
Deborah Golino, UC Davis	Science of Sustainable Viticulture

American Society for Enology and Viticulture
PO Box 1855
Davis, CA 95617-1855 USA
Tel: 530 753-3142 Fax: 530 753-3318
Email: society@asev.org Website: www.asev.org
Office located at 1784 Picasso Avenue, Suite D, Davis, CA 95616

Alphabetical Listing of Presenting Authors: Session Presentations and Enology and Viticulture Posters

Abstracts for session presentations are in the approximate order as noted in the conference program. There are no abstracts for the Warm Climate Symposium, keynote speakers, and merit and honorary research lectures.

Anthocyanin Tannin Reactions Observed with Labeled Malvidin-3-Glucoside

Andrew L. Waterhouse* and Alejandro Zimman

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[http://waterhouse.ucdavis.edu; fax: 530-752-0382]

A labeled anthocyanin (malvidin-3-glucoside) was added to a fermenting juice or to a young wine in order to understand the formation of pigmented tannins. For the addition to the wine, the reaction products were analyzed for color and tritium incorporation by liquid extraction with iso-amyl alcohol or by liquid chromatography. Results indicate that in an aged wine it is possible to find most of the anthocyanins incorporated into polymeric structures. The radioactive tracing also allows monitoring the precipitation of polymers, which leads to color loss and reduction of astringency. Variables such as wine pH, additional tannin levels, or copigmentation have small effects; however, oxygen exposure and temperature had large effects in the course of polymeric pigment formation. During fermentation there is a rapid disappearance of tritium from solution 24 hours after the addition, indicating that half of the anthocyanins are associated with grape solids at this point of the fermentation. In addition, the decline of malvidin-3-glucoside at late stages of fermentation does not correlate with a decrease of tritium in solution, indicating that chemical transformation of anthocyanins is taking place.

* indicates corresponding author

Effect of Cofermentation of Red Grapes with Different Amounts of White Skins on the Color of Young Red Wines

Maria P. Diago-Santamaria and Roger B. Boulton*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0900; email: rbboulton@ucdavis.edu]

The presence of a limited amount of white skins in red juice fermentations may lead to higher levels of phenolic compounds capable of behaving as copigmentation cofactors by capturing additional anthocyanins and building copigmentation stacks, which are highly colored. Two pairings of red and white grape varieties, Pinot noir with Pinot gris, and Sangiovese with Malvasia bianca, respectively, were selected to conduct several cofermentation trials at different levels of white skins additions. Separate lots of Pinot noir grapes with 0, 5, and 10% (w/w as grapes) of Pinot gris skins were fermented at commercial scale, whereas separate lots of 5 gallons of Sangiovese grapes containing 0, 4, 8, and 12% (w/w as grapes) of Malvasia bianca skins were microvinified under controlled conditions. In both studies, temperature and Brix were monitored along fermentation, and juice samples from each lot were collected daily and kept refrigerated until further analysis. Phenolic profile and composition of the musts and wines was accomplished by HPLC, and these values correlated by PLS with the spectrophotometric results obtained by other analytical methods: Folin-Ciocalteu for total phenols and copigmentation assay and Harbestson and Adam's assay for copigmented color, total tannins, and polymeric pigment. The stability of the color across the wines, as well as the changes in the major monomeric phenols and pigments were also quantified six months after fermentation. Significant differences in phenolic composition and color exhibition were encountered at specific levels of white skins additions in both trials. The amount of white skins added (at low loadings of white skins, two effects are expected to compete: the increase of copigmentation cofactors and the pigment adsorption on the skins) and the effect of the pairing of the varieties involved in the contributions of copigmentation and polymeric pigment to the color of the resulting wines will be discussed.

Seed and Skin Proanthocyanidin Extraction during Wine Production

Catherine Peyrot des Gachons and James A. Kennedy*

Department of Food Science and Technology, 100 Wiegand Hall
Oregon State University, Corvallis, Oregon, 97331
[Fax: 541-737-1877; email: James.Kennedy@oregonstate.edu]

Skin proanthocyanidins differ from seed proanthocyanidins in terms of composition, average degree of polymerization, and extractability. It is expected that their organoleptic properties differ as well. Understanding the exact influence of winemaking on skin and seed proanthocyanidin extraction is therefore important. Several authors have investigated differences in extraction by comparing proanthocyanidin amounts in wine made with or without pomace contact by the addition of supplementary quantities of seed or seed and stem tissues. These types of studies are time-consuming, difficult, and approximate. A method for determining the seed and skin proanthocyanidin proportion directly in wines has been developed. Analysis of proanthocyanidin cleavage products after acid catalysis in the presence of excess phloroglucinol led to two observations: first, the seed and skin proanthocyanidin extension subunit compositions were considerably different; second, their composition did not vary with extraction time. By comparing the proportional subunit composition in wine relative to the proportional subunit composition in corresponding grape seed and skin, it was possible to determine the contribution of each to wine. This procedure was used to investigate the extraction of proanthocyanidins during fermentation under various winemaking conditions to investigate how different winemaking operations affect the proportion of skin and seed proanthocyanidins in wine.

*** indicates corresponding author**

Anthocyanin Composition of Table Grape Cultivars

M. Cecilia Peppi and Nick K. Dokoozlian*

Department of Viticulture and Enology, University of California, Davis
and the U.C. Kearney Agricultural Center, 9240 South Riverbend Ave., Parlier, CA 93648
[Fax: 559-646-6593; email: nkdokoozlian@uckac.edu]

The anthocyanin composition of 20 commercially important table grape cultivars was determined using reversed-phase HPLC. As expected, table grape cultivars contained each of the pigments commonly found in *Vitis vinifera* cultivars: delphinidin, cyanidin, petunidin, peonidin, and malvidin 3-glucosides, as well as the acylated forms of these pigments. Pigment composition varied significantly among cultivars, with cyanidin and peonidin found to be the primary pigments of most red cultivars, including Flame Seedless, Redglobe, and Crimson Seedless. The primary pigment of black table grape cultivars, such as Ribier, Fantasy Seedless, and Autumn Royal, was malvidin-3-glucoside. Large differences in total skin pigment content were found among cultivars with similar anthocyanin compositions. Peonidin represented a significant portion of the total pigment in both Crimson Seedless and Flame Seedless, but Flame Seedless accumulated greater amounts of pigment. This suggests that the regulation of the pathways responsible for each pigment varies among cultivars and that pigment composition is not a good indicator of a cultivar's coloring potential. Although large differences in total pigment accumulation were observed due to growing region, crop types, and cultural practices, anthocyanin composition remained relatively constant. Crimson Seedless in Davis (region IV) and Parlier (region V) exhibited similar anthocyanin compositions, although the total pigment content of fruit from Davis was significantly greater. Similarly, primary and second crop clusters of both Redglobe and Crimson Seedless had similar anthocyanin compositions, although the second crop clusters of both cultivars were more highly pigmented. Gibberellic acid and forchlorfenuron significantly reduced total pigment accumulation in several red cultivars, but had little effect on overall pigment composition.

Genomic Tools for the Enhancement of Stress Tolerance in *Vitis vinifera*

Grant R. Cramer,* Fadi Alkayal, Elizabeth A.R. Tattersall, Chunhong Mao, and John C. Cushman

Department of Biochemistry, University of Nevada, Reno, NV 89557
[Email: cramer@unr.edu]

Abiotic stresses affect important aroma, flavor, and color constituents by altering metabolite composition. 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, which is important in arid regions like Nevada. In order to understand how growth is affected and wine-quality improvements might arise following abiotic stress exposure, we have initiated an expressed sequence tag (EST)-based gene discovery program focused solely on abiotically stressed plants. cDNA libraries were constructed from mRNA isolated from leaf, root, and berry tissues of *Vitis vinifera* cv. Chardonnay exposed to various abiotic stress conditions. To date, we have sequenced over 20,000 ESTs and anticipate completing another 30,000 sequences. Raw sequence data were processed through an automated EST analysis pipeline (ESTAP) developed at the Virginia Bioinformatics Institute (Blacksburg) in collaboration with UNR and S.R. Noble Foundation (Ardmore, OK). Initial sequence analysis reveals approximately 50% novel or unknown genes and a low redundancy of transcripts. Approximately 13% of the known genes are associated with disease, defense, and stress response functions. All unique EST data generated to date have been deposited in GenBank and are available to the public. A unigene set has been assembled and used in the construction of an oligonucleotide microarray in collaboration with the International Grape Genomics Program. The scientific community will use the microarrays for global transcript analysis to elucidate gene function in plant development and responses to the environment.

* indicates corresponding author

Extended Genetic Linkage Map of a *Vitis rupestris* and *Muscadinia rotundifolia* Population and Comparisons with the International *Vitis vinifera* Reference Map

Summaira Riaz and M. Andrew Walker*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email:awalker@ucdavis.edu]

We report on the construction of an extended framework linkage map based on an F2 population derived from a cross of two *Vitis rupestris* x *Muscadinia rotundifolia* siblings. The map was constructed with sequence repeat (SSR), amplified fragment length polymorphism (AFLP), and expressed sequence tag polymorphism (ESTP) markers. The mapping population segregates for resistance to *Xylella fastidiosa*, the bacterial causal agent of Pierce's disease, and for resistance to *Xiphinema index*, the dagger nematode vector of grapevine fanleaf virus. The original map of this population consisted of 116 individuals. The expanded mapping population contains 181 plants. To date, a total of 210 SSR markers and 63 grape EST sequences have been tested; 127 SSR and 14 ESTP markers were polymorphic for this population. Fifty-four EcoRI and MseI primer combinations from the previous map will be applied to the extended population to obtain 216 framework AFLP markers for maternal and paternal parents. We also report on estimates of genome size, expected genome coverage, and observed genome coverage based on SSR and AFLP markers. SSR markers common to both parents will be used to estimate recombination rate differences between maternal and paternal parents. Comparisons of common SSR markers are made between this map and the international *Vitis vinifera* reference map to identify potential differences based on grape species.

Phenolic Biosynthesis-related Gene Expression in Grapevine Leaves

Phanikant V. Turlapati, Hesheng Hou, Wenping Qiu, and László G. Kovács*

Department of Fruit Science, Southwest Missouri State University
9740 Red Spring Road, Mountain Grove, MO 65711
[Fax: 417-926-6646; email: LaszloKovacs@smsu.edu]

Phenolic metabolites have been implicated in the ability of plant tissues to defend themselves from pathogens. In grapevine, there is evidence that the inducible accumulation of certain phenolic metabolites, most notably stilbenes, enhances resistance to fungal pathogens. Little is known, however, about the role of constitutively formed phenolics in disease resistance. To address this question, the expression of genes that encode enzymes in the phenylpropanoid and flavonoid biosynthesis pathways in grapevine leaves was studied. We explored the expression of these genes in *Vitis vinifera* and *Vitis aestivalis*, a disease-susceptible and a highly disease-resistant grapevine species, respectively. We cloned cDNAs and identified expressed sequence tags (ESTs) of genes that direct phenolic biosynthesis from leaves of *V. aestivalis* var. Norton. Based on ESTs present in our database and in publicly available *V. vinifera* databases, we established a preliminary metabolic map of phenolic biosynthesis in grapevine leaves. We are also testing the hypothesis that certain phenolic pathway-related genes may be differentially expressed in *V. aestivalis* and *V. vinifera.* Currently, we are extracting RNA from healthy leaves of both species and comparatively assessing the expression levels of individual genes in Northern hybridization experiments. As phenolic biosynthesis enzymes are regulated primarily at the level of transcription, the relative abundance of specific mRNAs will be indicative of the activity of the corresponding pathways.

*** indicates corresponding author**

Defense-related Genes in *Vitis aestivalis* var. Norton

Xueyan Zhong, Hesheng Hou, Laszlo Kovacs, and Wenping Qiu*

Department of Fruit Science, Southwest Missouri State University
Mountain Grove, MO 65711
[Fax: 417-926-6646; email: weq070f@smsu.edu]

Norton grapevine (*Vitis aestivalis* var. Norton), a cultivated variety of *V. aestivalis*, is highly resistant to most severe fungal diseases. In order to understand the genetic mechanism underlying the grapevine disease resistance, a cDNA library from young leaf tissues of Norton grapevine for identifying defense-related genes was constructed. Preliminary functional annotation of nonredundant expressed sequence tags has identified those that aligned with defense-related genes, including Avr9/Cf-9 rapidly elicited protein 65, proline-rich protein, basic chitinase, pathogenesis-related (PR) protein-5, S-adenosyl-L-methionine synthetase, flavanone 3-hydroxylase, and chalcone synthase. To perform a comparative genomics study of defense-related genes, we are compiling the plant defense-related genes, including resistance genes, PR protein genes, and genes involved in the hypersensitive response and systemically acquired resistance pathways and in the synthesis of antimicrobial peptides. Defense-related orthologous genes of *V. aestivalis* var. Norton, *V. vinifera*, and other plant species will be comparatively studied. We also analyzed the expression profiles of selected defense-related genes in both disease-resistant Norton and disease-susceptible *V. vinifera*-derived grapevines by RNA-blot assays. These studies should lead to the discovery of candidate genes that determine the resistant trait in grapevine and further our understanding of grapevine-pathogen interactions.

Vegetal Aroma Descriptors and Related Chemical Compounds

Leslie D. Preston, David E. Block, Nick K.Dokoozlian, Ann C. Noble, Hildegarde Heymann, John H. Thorngate, and Susan E. Ebeler*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email:seebeler@ucdavis.edu]

Cabernet Sauvignon is the most notable variety plagued by vegetal aroma characteristics. The bell pepper aroma associated with Cabernet Sauvignon is attributed to the compound 3-methoxy-2-isobutylpyrazine (MIBP), and extensive research has been conducted on the viticultural practices linked to increased levels of MIBP in grapes. However, the general vegetal term can also be applied to a wide range of aromas and chemical compounds. This project seeks to broaden our understanding of what aroma attributes fall within the vegetal category and, subsequently, to link that array of aroma descriptors to potential chemical sources other than MIBP. Descriptive analysis of 16 Cabernet Sauvignon wines was compared to the sensory findings of an "expert" panel, comprised of winemakers and enologists from California. While the descriptive panel focused on specific aroma attributes, including eucalyptus, bell pepper, olive, and cooked vegetable, the expert panel was asked to sort the same wines, initially according to their chosen criteria and then according to high, medium, and low levels of vegetal aroma and sulfur defects. A comparison of the descriptive panel data and the expert panel sortings revealed a clear overlap in the classification of vegetal aroma for some, but not all, of the wines. In an effort to understand what distinguishes these notably vegetal wines, a basic chemical profile of all wines is being developed using GC and GC-MS, with the results compared to the sensory data.

* indicates corresponding author

Influence of Viticultural Practices on the Aroma of 2001 Napa Valley Cabernet Sauvignon Wines

Hua Chen and David E. Block*

Department of Viticulture and Enology, University of California, Davis
One Shields Avenue, Davis, CA 95616
[Tel: 530-754-6046; fax: 530-752-0382; email: deblock@ucdavis.edu]

To reveal the relationship between simultaneous multiple viticultural practices and the aroma characteristics of final wines, 12 viticultural practices (vineyard block, rootstock, vine density, shoot number, trellis, overall water, orientation, vine age, fertilization [N and K], and pruning method) were varied using existing Cabernet Sauvignon viticultural trials at the Oakville Experimental Vineyards, Napa, CA. In all, 38 combinations of treatments were chosen. The grapes from each lot were harvested at 24.0 Brix (±0.4) in the fall of 2001 and identically processed into the final wines in the UC Davis research winery. After 10 months of aging, a descriptive analysis was performed on these wines, and 10 aroma attributes (cocoa, cherry, berry, dried fruit, vanilla, green pepper, cooked veggie, olive, pepper, and mushroom) were rated by a trained panel of judges. Intensity scores of these attributes were further analyzed by multivariate statistical analysis. The effects of the viticultural treatments on sensory characteristics of the wines were examined and a comparison was made to a similar analysis completed for wines from the previous vintage.

Canopy Density and Cluster Location Interact on Berry Microclimate and Fruit and Wine Composition of Cabernet Sauvignon Grown in Four Regions of California

Joseph P. Cotta and Nick K. Dokoozlian*

Department of Viticulture and Enology, University of California, Davis
and the U.C. Kearney Agricultural Center, Parlier, CA 93648
[Fax: 559 646-6593; email: nkdokoozlian@uckac.edu]

Effects of canopy density and cluster location on the fruit microclimate and composition of Cabernet Sauvignon were examined in four premium winegrowing regions of California (Napa, Lodi, Gonzales, and Paso Robles) ranging in mean daily temperature. Canopy density in the fruiting zone was manipulated following berry set by varying the amounts of basal leaf and lateral shoot removal. Treatment effects on the afternoon sun (south or west) and afternoon shade (north or east) portions of the fruiting zone were measured separately. A negative, near linear relationship was found between leaf layer number (LLN) in the fruit zone and the percentage of total clusters located on the canopy exterior. Berry temperature and cluster sunlight exposure increased as LLN in the fruiting zone declined in all regions. Berry weight was generally least for fully exposed clusters and greatest for shaded clusters. Soluble solids were generally lowest in fully exposed berries and increased slightly as LLN increased. Titratable acidity and malic acid content generally declined as cluster exposure increased, while juice pH increased. However, these trends were reversed for fully exposed clusters, where elevated fruit temperatures likely delayed fruit ripening. Treatment effects on skin anthocyanin content generally reflected differences in berry temperature due to sunlight exposure. In all growing regions, fully exposed clusters on afternoon sun side of the canopy had lower anthocyanin concentrations compared to partially shaded clusters. Chemical and sensory analyses of wines produced from each location were generally well correlated with the above-mentioned trends in fruit composition.

* indicates corresponding author

Environmental Control of Phenolic Biosynthesis in Grape Cell Suspensions

Mark Krasnow and Terence Murphy*

Section of Plant Biology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-5410; email:tmmurphy@ucdavis.edu]

Grapes synthesize a variety of phenolic compounds important to winemaking, including anthocyanins, stilbenes, and tannins. All of these compounds come from one branched biochemical pathway. We examined how different growth conditions affect phenolic accumulation, as well as activities of key enzymes in the phenolic pathway, in cell cultures of *Vitis vinifera* (cv. Gamay Freaux). We have identified treatments that differentially affect the accumulation of anthocyanins and stilbenes, as measured by HPLC. Growing the cells in darkness leads to a general decrease in the anthocyanins but also affects the ratio of acylated to nonacylated pigments. Treatment of the cells with the stress hormone methyl jasmonate increases all measured phenolics in a dose-dependent manner, but does not affect the relative amounts of the compounds. The activity of phenylalanine ammonia-lyase, the first enzyme in the pathway, is induced by methyl jasmonate, although the induction is not of the same magnitude as the observed increase in phenolic compounds. Growth in the dark does not affect the activity of this enzyme. We are examining other enzyme activities (dihydroflavonol reductase, UDPG-flavonoid glucosyltransferase) to determine which enzymes are important to the control of phenolic production and how these enzymes are affected by differing growth conditions. Information from cell cultures should help optimize cultural practices in the vineyard to produce fruit with the desired phenolic composition.

Phenolic Characterization of Zinfandel Fruit and Wine: Tannin and Phenolics from Veraison to the Bottle

Matthew S. Reid and Douglas O. Adams*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email: doadams@ucdavis.edu]

The phenolic content of fruit of two clones of *Vitis vinifera* L. cv. Zinfandel was studied from veraison through harvest at a single vineyard site during the 2002 growing season. Phenolic composition was measured in fruit during ripening and through the first racking of the resulting wines. Differing patterns of change in berry skin and berry seed phenolic composition were observed in the two different clones during ripening, indicating that clonal selection is important to phenolic composition. Postpress pomace analysis revealed that skin tannin and phenolics extraction were comparable but that the extraction of small seed phenolics far surpassed that of seed tannin. The differential extraction of seed and skin tannin and phenolics indicates that knowledge of berry phenolic content alone is insufficient for predicting wine phenolic composition. However, awareness of the differing phenolic extraction characteristics of seeds and skins might help winemakers achieve the desired phenolic profile in finished wines and thus reduce the need of fining to adjust levels before bottling. We also analyzed the phenolic composition of 200 commercial varietal Zinfandel wines to characterize the phenolic profiles of the finished products. The results of the finished wine survey show that the phenolic profile of commercial Zinfandel wines is highly variable. The total phenolic content of the wines analyzed varied by a factor of 4, while tannin content varied by a factor of 13.

*** indicates corresponding author**

Impact of Biotin and Pantothenic Acid on Fermentations Induced by *Saccharomyces*

C.G. Edwards,* X.D. Wang, and J.C. Bohlscheid

Department of Food Science and Human Nutrition, Washington State University
Pullman, WA, 99164
[Fax: 509-335-4815; email: edwardsc@wsu.edu]

The impact of assimilable nitrogen and vitamins (biotin and pantothenic acid) on fermentation rate and synthesis of volatile compounds by *Saccharomyces* under fermentative conditions was studied. Factorial designs were employed with the concentrations of yeast assimilable nitrogen (YAN) (60 and 250 mg/L), biotin (0, 1, and 10 µg/L), and/or pantothenic acid (10, 50, and 250 µg/L) as variables. In general, fermentations did not reach dryness and yeast growth was very poor in media without any biotin. However, the rate of fermentation and yeast growth seemed to be more dependent on the amount of assimilable nitrogen in the medium than on biotin. The concentration of the vitamin did influence production of H_2S and other volatiles such as the undesirable higher alcohols (isobutyl and isoamyl alcohols) and ethyl esters. In media containing 250 µg/L pantothenic acid, H_2S production by two different species of *Saccharomyces* decreased when YAN was increased from 60 to 250 mg/L. Conversely, H_2S production was significantly higher when the concentration of assimilable nitrogen was increased if pantothenic acid was deficient (10 or 50 µg/L). Yeast synthesis of other volatile compounds was also impacted by both assimilable nitrogen and pantothenic acid. While growth and fermentative rate of *Saccharomyces* was influenced more by nitrogen than by vitamins, complicated interactions exist between these nutrients that affect the synthesis of volatile compounds including H_2S.

Effect of Temperature on Yeast Growth and Performance in Normal and Problem Wine Fermentations

Russell Fish and David E. Block*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax 530-752-0382; email: deblock@ucdavis.edu]

Stuck and sluggish fermentations are an ongoing and sometimes perplexing problem in the wine industry. Although a significant amount of research has been performed in determining causes for problem fermentations, temperature effects have not been thoroughly studied. Our recent work with commercial fermentations indicates, however, that temperature is perhaps the most important factor in determining fermentation kinetics. A mechanistic model for prediction of fermentation kinetics was developed and successfully used to predict fermentation performance based on initial juice composition. The purpose of this project was to investigate the role of temperature in problem fermentations and expand the mechanistic model to include temperature effects so that better predictions could be made. Small-scale fermentations were performed in duplicate at 11, 15, 20, 25, 30, and 35°C. The nitrogen and sugar levels in these fermentations were also varied so that fermentation kinetics would be normal, sluggish, or stuck. Throughout all fermentations, samples were taken and analyzed for total cell count, viability, ammonia, amino acids, sugars, and ethanol. Nonlinear regression was used to fit parameters of the mechanistic model to the data in order to find the temperature dependence of each parameter. The significance of the dependence of each of the parameters on temperature and the potential applications of this analysis to fermentation management will be discussed.

* indicates corresponding author

Effect of Temperature on Genomic Gene Expression in Wine Yeast

Staci Nugent, Jeff Mangahas, and Linda Bisson*

Department of Viticulture and Enology, University of California, Davis
One Shields Av., Davis, CA 95616
[Fax: 530-752-0382; email: lfbisson@ucdavis.edu]

A primary cause of fermentation arrest during wine production is exposure to extremes of temperature. In order to understand the biological pathways that are involved in yeast adaptation to temperature, fermentations were performed with Côte des Blancs (*Saccharomyces cerevisiae*) and Premier cuvee (*S. bayanus*) strains at 25 and 35°C, followed by analysis of genomic gene expression using DNA chips in samples extracted at log and stationary phase. Differences were found in the patterns of gene expression in the two strains. The heat shock genes, which play crucial roles in the adaptive response of yeast to stress, were induced to a much greater extent in the high-temperature Premier cuvee fermentations. In addition, at the higher temperature during stationary phase, the expression of genes important in cell wall biogenesis, cytoskeleton, and cytokinesis pathways was generally increased in Premier cuvee compared to Côte des Blancs fermentations; these results suggest that Premier cuvee is more actively growing at the higher temperature. To further elucidate how wine yeast strains adapt to sudden changes in temperature, we are currently conducting genomic gene expression analysis on samples from fermentations of other strains of *S. cerevisiae* (Montrachet and French Red) and *S. bayanus* (Uvaferm 43) that included a mid- or late-temperature shift from 25 to 30°C to induce heat stress.

Hyperosmotic Stress Response of *Saccharomyces cerevisiae* to Icewine Juice

Gary Pigeau and Debra Inglis*

Cool Climate Oenology and Viticulture Institute, Brock University
500 Glenridge Ave., St. Catharines, Ontario, Canada L2S 3A1
[Fax: 905-688-3104; email: dinglis@spartan.ac.brocku.ca]

Salt-induced hyperosmotic stress on yeast cells evokes a high osmolarity glycerol (HOG) response, causing yeast to produce glycerol within the cell to balance the external osmotic pressure. This response is required for yeast survival and is dependent upon expression of genes encoding enzymes involved in glycerol synthesis, such as glycerol-3-phosphate dehydrogenase (*GPD1*). In order for yeast to continue to produce glycerol, the cofactor required for glycerol synthesis, NADH, must be regenerated. Three cytosolic aldehyde dehydrogenases (*ALD*s) in *Saccharomyces cerevisiae* can oxidize acetaldehyde to acetic acid, two of which are NAD+ dependent and may be linked to NADH regeneration. This project investigated wine yeast fermenting high sugar juices to determine whether an analogous HOG response occurs to counteract the osmotic pressure exerted by fermentable sugars. We previously found that yeast fermenting icewine juice at 40 Brix produce about 10 times the acetic acid and two times the amount of glycerol compared to that found in table wine fermentations, but the metabolic pathways responsible are not known. Gene expression patterns of *ALD3, ALD6,* and *GPD1* were measured throughout the fermentation of high sugar juices (40 Brix) and diluted icewine juice (20 Brix) and were correlated to acetic acid and glycerol concentrations in the wines. It was determined that *ALD6* was expressed at similar levels across the fermentations and that *GPD1* and *ALD3* were expressed 2-fold and 30-fold greater during the high sugar fermentations, with a 2-fold and 8-fold increase in glycerol and acetic acid concentrations, respectively.

*** indicates corresponding author**

Low Glucose Utilization in *Saccharomyces cerevisiae*

Vidhya Ramakrishnan, Viyada Kunathigan, and Linda F. Bisson*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis CA 95616
[Tel: 530-752-3835; Fax: 530-752-0382; email: lfbisson@ucdavis.edu]

The ability of *Saccharomyces cerevisiae* to adapt to different sugar concentrations allows consumption of all of the available fermentable sugar. Stuck fermentations arise when the yeast are not able to complete the adaptive process. Several genes involved in adaptation to low glucose utilization have been discovered. One of these genes is encoded by *SNF3*. Null mutants of this gene are unable to adapt rapidly to low sugar concentrations. Transcriptome analyses were performed on *SNF3* wild type and mutant strains to define the pathways that differ. The wild type strain undergoes a specific adaptive response to low glucose that is absent in the mutant, as revealed by the analysis of the functional gene families expressed differentially. In contrast, the mutant is unable to "see" the low glucose concentration and enters a metabolically quiescent resting phase, as in fermentation arrest in wine production. In order to better understand the adaptation process, mutations allowing the cells to resume fermentation of glucose in a *snf3* null background were isolated and labeled "*rgg*" for *r*estoration of *g*rowth on *g*lucose." Transcriptome analyses were also conducted on the *rgg* mutants to understand the mechanism by which sugar utilization is restored. Some of these mutations were downstream of the *SNF3* sensor molecule and restore growth by creating a constitutive and false signal that low glucose is present. A more interesting class of mutation impacted energy efficiency. Two mutations decreased conversion of glucose to storage carbohydrate, thereby increasing fermentation.

Proteomic Markers of Normal Fermentations

Paula A. Mara, Kathryn C. Weiss, Christopher D. Cooney, and Linda F. Bisson*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Tel: 530-752-3835; Fax: 530-752-0382; email: lfbisson@ucdavis.edu]

Yeast activate and produce sets of enzymes and other protein markers based on fermentation conditions. The goal of this research was to identify markers that may be used to distinguish between normal and problem fermentations. Laboratory scale fermentations were conducted, using commercially available wine yeast strains. Samples were collected and analyzed for protein expression and transcriptional activity under normal and problem fermentation conditions. 2D-PAGE, silver staining, and proteomics software were optimized and used to map and quantify protein expression. Genomic RNA analysis was also conducted to identify protein transcription. Proteins of interest from the 2D-PAGE analysis and the genomic RNA analysis were identified using MALDI-TOF analysis. Typical patterns of yeast protein markers were identified for normal fermentations and compared with the patterns identified in problem fermentations. Similarities and differences between these patterns can guide the search for markers that can be used to distinguish between normal and problem fermentations.

*** indicates corresponding author**

Effect of Training and Pruning Practices on Eutypa Dieback Control in Cabernet Sauvignon Grapevines

Sanliang Gu,* Robert Cochran, Kenneth Fugelsang, Chuck Ingles, John Ledbetter, and Paul Verdegaal

Viticulture and Enology Research Center, California State University, Fresno
2360 East Barstow Ave., Fresno, CA 93740
[Fax: 559-278-4795; email: sanliang@csufresno.edu]

Eutypa dieback is one of the most destructive fungal diseases of woody tissues in grapevines. The pathogen enters primarily via pruning wounds during the rainy dormant season when spores are released from the infected wood. It is hypothesized that reduced exposure of pruning wounds to infection by altering training-pruning practices results in lower disease development. The study was conducted with Cabernet Sauvignon/Freedom grapevines planted in 1992. Experiment design was a split-plot factorial of two soil types and six training-pruning systems with three replications. Vines were evaluated for symptomatic shoots, dead spurs, and dead arms when shoots were 10 to 15 inches long. Disease incidence and severity were expressed as the percentage of vines showing symptoms and the number of symptomatic shoots, dead spurs, or the length of dead arms per symptomatic vine or per acre, respectively. Disease symptoms were first found in 1996 and continued to increase in the vineyard. Training-pruning practices affected disease development considerably. In 2002, vines grown on the higher capacity soil had greater incidence and severity of symptomatic shoots but lower incidence and severity of dead spurs and dead arms. Vines trained to bilateral cordon and Sylvoz with hand spur pruning displayed greatest incidence and severity of Eutypa dieback. Head-trained vines with hand cane pruning or mechanically pruned vines displayed lower level of Eutypa dieback development. The incidence and severity of Eutypa dieback was the lowest in minimally pruned vines. Soil fertility and training-pruning practices interacted only to affect the severity of symptomatic shoots.

Progress on the Development of Nematode Resistant Rootstocks at UC Davis

M. Andrew Walker,* Daniel Ng, Liang Zheng, and Howard Ferris

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email:awalker@ucdavis.edu]

Several factors have dramatically increased the need for grape rootstocks with broad and durable nematode resistance, including the evolution of more aggressive strains; the lack of fallow and rotation, encouraging this evolution; the relative ineffectiveness of nematicides on deeply rooted perennial crops; and environmental concerns about the use of these pesticides. A breeding program to develop and introduce nematode resistant rootstocks has advanced on two fronts: resistance to fanleaf degeneration and broad resistance to root-knot nematodes. Selections from a series of crosses among *Vitis rupestris* x *Muscadinia rotundifolia* selections have excellent resistance to *Xiphinema index* and they have been tested at fanleaf sites for up to six years. Vine performance and infection levels at these trials will be discussed. The best of these selections have been crossed to commercial rootstocks to improve horticultural characters. About 5,000 seedlings from crosses among *V. candicans*. *V. champinii*, *V. cinerea*, *V. riparia*, *V. rufotomentosa,* and *V. rupestris* were tested for ease of rooting. Those that rooted well were challenged by *X. index, Meloidogyne incognita* Race 3, and two aggressive strains, *M. incognita* Harm C and *M. arenaria* Harm A, in potted vine tests. Thirteen selections have excellent resistance against all four nematodes. Five of these have complete resistance when challenged concurrently by all these nematodes. Four selections also have at least moderate resistance to *Mesocriconema xenoplax*, ring nematode. Rootstock trials with these selections began in spring 2002.

* indicates corresponding author

Pierce's Disease Resistance: Performance of Field Resistant Cultivars under Greenhouse Screening Conditions

Alan F. Krivanek and M. Andrew Walker*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email:awalker@ucdavis.edu]

Pierce's disease (PD), a bacterial disease caused by *Xylella fastidiosa* (Xf), has been an increasing concern to the California viticulture industry. Development of PD resistant varieties can provide a long-term solution to this disease. Evaluation of grape genotypes for resistance needs to correlate well with past field resistance data, and screening procedures should be rapid in order to be a useful tool in PD genetics studies or breeding programs. A resistance screening procedure was evaluated on five southeastern United States cultivars in order to define more fully the general response of known field resistant genotypes to Xf. Four or five randomized replicates of Roucaneuf (SV12.309), D'Arpa, Blanc Du Bois, Zehnder 71-50-1, and Tampa were inoculated twice with the Stags Leap Xf isolate along with two known field-susceptible cultivars, Chardonnay and Chenin blanc. Following inoculation, each plant was grown under greenhouse conditions for 12 weeks. Four tissues were sampled, cane internode, node, petiole, and leaf blade, from eight positions along the inoculated canes. Bacteria numbers were measured in each sample using an optimized ELISA procedure. Results and implications for PD screening procedures will be discussed.

Evaluation of Tolerance to Pierce's Disease and Botrytis in Transgenic Plants of *Vitis vinifera* L. Expressing the Pear *pgip* Gene

Cecilia Aguero,* Abhaya Dandekar, and Carole Meredith

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email: cbaguero@ucdavis.edu]

Polygalacturonase-inhibiting proteins (PGIP) are leucine-rich repeat plant cell wall proteins that specifically inhibit fungal polygalacturonases (PGs). Their role in plant defense response suggests that they may be useful for genetic engineering to obtain transgenic plants with increased tolerance to fungal infection. In addition, the fact that *Xylella fastidiosa*, the causal agent of Pierce's disease (PD) in grapevines, has genes putatively encoding PG and other cell wall-degrading enzymes led us to the hypothesis that PGIP could confer tolerance against this bacterium. To test this hypothesis, proembryogenic calluses originating from anthers of *Vitis vinifera* cvs. Thompson Seedless and Chardonnay were cocultivated with *Agrobacterium tumefaciens* strain EHA 101 harboring binary plasmid pDU94.0928 that contains the pear *pgip* gene under the control of the CaMV 35S promoter. Plants from 49 independently isolated lines were transferred to the greenhouse. Putative transgenic lines growing in the greenhouse were analyzed by PCR, Western blotting, and PGIP activity assays. Western blot analysis demonstrated the presence of the protein in roots, leaves, and young stems of the transgenic plants but not in untransformed controls. High levels of enzyme activity were found in crude extracts from leaves and in xylem sap of transgenic lines obtained from independent transformation events but not in untransformed controls. Preliminary results have shown that development of PD in transgenic lines analyzed thus far is delayed. In addition, lesion expansion was slowed in leaves of transgenic grapes infected with *Botrytis cinerea*. Results regarding grape transformation with different green fluorescent protein marker gene constructs will be presented.

* indicates corresponding author

Rapid Method for Determining Free Sulfur Dioxide in Red Wine

Mee Godard and James A. Kennedy*

Department of Food Science and Technology, 100 Wiegand Hall
Oregon State University, Corvallis, OR 97331
[Fax: 541-737-1877; email: James.Kennedy@orst.edu]

Sulfur dioxide is a preservative used in wine. The most commonly used analytical method in commercial wineries for determining sulfur dioxide in red wine is the aeration oxidation method. This method requires standardized solutions, analytical skills, and is time-consuming when many wines are analyzed. A method developed by Burroughs is based on the equilibrium between sulfur dioxide and anthocyanins. We optimized this method and determined its reproducibility, ruggedness, and relationship to the aeration-oxidation method. The correlation factor between aeration oxidation and this colorimetric method for free sulfur dioxide was $r^2 = 0.959$. The relative standard deviation for repeatability was 2.4%. This method has several advantages as it is rapid, able to run several samples simultaneously, and does not require major skills or standardized solutions.

Comparison of Tannin-Binding Capacity of Salivary Proteins and Bovine Serum Albumin in a Protein Precipitation Assay

James F. Harbertson, Jeremy Weintraub, Ann C. Noble, and Douglas O. Adams*****

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email: doadams@ucdavis.edu]

Tannins are the most abundant class of phenolics found in grapes and are also the primary source of astringency in red wine. Tannin is thought to cause astringency by precipitating salivary proteins that normally lubricate the surfaces of the mouth. Saliva contains proline rich proteins (PRP) and a-amylase. The main biological function of PRPs in the mouth is believed to be tannin precipitation due to their high tannin-binding capacity. We have been using the protein precipitation method to measure tannin in grapes and wine that uses bovine serum albumin (BSA) as the tannin-binding component. To determine if the values measured by the BSA tannin coprecipitation method were related to salivary protein tannin coprecipitation, the tannin-binding capacity of salivary proteins and BSA were compared. Saliva was collected from the parotid glands of eight human subjects via the Stenson's duct. The tannin-binding capacity of each protein was determined by adding increasing amounts of saliva or BSA to a reaction mixture containing a fixed amount of red wine. BSA and salivary protein were found to have different tannin-binding capacities, as reported by others. However, under conditions where protein is in excess, the amount of tannin bound by BSA and salivary protein was equal. Thu, tannin determinations using the BSA tannin coprecipitation method, where protein is deliberately in excess, measure the same amount of tannin in a wine that would bind to salivary proteins, suggesting that the BSA tannin-binding assay measures the amount of tannin that causes astringency.

*** indicates corresponding author**

Effects of Low-Level Oxygenation in Winemaking on Phenolic Constituents

Felipe Laurie and Andrew L. Waterhouse*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Email: alwaterhouse@ucdavis.edu]

The use of commercial devices to introduce small amounts of oxygen into wines in production stages has become popular in the last few years. Beneficial effects, such as "softer tannins" and color stabilization among others, have been claimed. The influence of different regimes of low-level oxygenation in winemaking on phenolic composition in commercial-scale trials was investigated. Oxygen flow-levels applied and dissolved oxygen values were measured with sensitive devices. To allow wine sampling and determinations of dissolved oxygen minimizing air-oxygen contamination, a sample circulation system was constructed using stainless steel tubing. The analysis of phenolic concentration and composition was followed up on a weekly and monthly basis (depending on the stage of the treatments) through both chromatographic and spectral methods, including normal phase HPLC, reversed-phase HPLC, Folin-Ciocalteu, copigmentation assay, and a protein precipitation assay for tannins and polymeric pigments. A tendency for significant losses of specific phenolic species (i.e., quercetin) and smaller changes for other individual and classes of phenolic compounds have been observed. Future studies will evaluate free radical levels and sensory effects.

Impact of Micro-oxidation on Vegetal Characters and Polymeric Color in Commercial Cabernet Sauvignon

Carl O. DiManno and David E. Block*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email: deblock@ucdavis.edu]

Wineries throughout Europe, Australia, and increasingly the United States treat wine with commercial micro-oxidation techniques. While commercial evaluations of treatment impact on sensory properties are somewhat common, systematic controlled studies of the effects of this technique on the chemical and sensory characteristics of the resulting wine have not been prevalent. In 2003, four wines with high and low perceived vegetal characters were treated as part of a micro-oxidation trial. Two Cabernet Sauvignon wines were treated in a Napa (CA) winery and two Merlot lots were treated in a Carneros (CA) facility. Portions of the Cabernet Sauvignon wines were treated in small-scale trials at varying oxidation rates. All lots were analyzed for vegetal components, including pyrazine, sulfur species, and hexanal. Other analyses included acetaldehyde and phenolic polymerization. Changes in pyrazine (bell pepper), sulfur species (cabbage, garlic, canned vegetable), and hexanal (grass) as a function of time and treatment rate determine whether these components are directly affected by micro-oxidation. Increases in acetaldehyde and phenolics structure indicate whether masking or changes in wine matrix account for the perceived reduction in vegetal characters from micro-oxidation. Results of the analyses will help to better understand the impact of oxidation and oxidation rate on color and vegetal contributors in red wine.

*** indicates corresponding author**

Population Dynamics of *Oenococcus oeni* Strains in Wine: Effect of SO_2 Addition and Yeast Inoculation

Cristina Reguant,* Ramon Carreté, and Albert Bordons

Department of Biochemistry and Biotechnology, Rovira i Virgili University, Tarragona
P. Imperial Tarraco, 1, Tarragona, 43005 Spain
[Fax: 34-977558232; email: reguant@quimica.urv.es; email through December 2003: mr295@cornell.edu]

Metabolic activity of *Oenococcus oeni* produces modifications in wine composition that vary depending on the strain. Thus, sensory qualities of wine are related to the *O. oeni* strains growing during the winemaking process. A novel method based on RAPD-PCR was used to follow the population dynamics of indigenous and inoculated *O. oeni* strains during alcoholic and malolactic fermentations in red wine. The effect of different SO_2 additions (0, 50, and 100 mg/L) and the use of a yeast starter culture on *O. oeni* populations were evaluated. Multiplex RAPD-PCR, using two primers instead of one in the same reaction, allowed us to detect the presence of up to 22 different *O. oeni* strains in the fermentations developed. A noticeable diversity of strains was observed in all conditions studied. In the inoculated malolactic fermentations, the main presence of the commercial *O. oeni* strain was confirmed. In the not inoculated fermentations, one of the indigenous bacteria dominated the population. Depending on the wine, different strains were found to be responsible for the spontaneous malolactic fermentations, showing a better adaptation of some strains to specific wine conditions. For the same SO_2 addition (50 or 100 mg/L), *O. oeni* strains were strongly inhibited when a specific yeast starter had been used, suggesting that yeast strain during alcoholic fermentation might play a role in the control of *O. oeni* indigenous populations.

Effects of Cover Crops on a Northern California Vineyard Ecosystem

Chuck A. Ingels,* Kate M. Scow, and Desley A. Whisson

University of California Cooperative Extension
4145 Branch Center Rd., Sacramento, CA 95827
[Fax: 916-875-6233; E-mail: caingels@ucdavis.edu]

Several cover crop mixes were planted for three years (1998 to 2000) in a winegrape vineyard in Sacramento County. The trial was conducted in a Merlot vineyard on a silt loam soil. Vines were planted in 1993 on 5BB rootstock, spaced 7 x 11 ft. Five mixes were used: California native perennial grass (no-till); annual clover (no-till); bell bean/vetch/pea (disked); barley/oat (disked); and disked control. Cover crops were planted on either side of entire rows, with a disked middle separating treatment replicates. A 4-to-5-ft herbicide strip was maintained under the vines. Drip irrigation and fertigation were applied uniformly across all treatments, and supplemental nitrogen fertilizer was applied to the grass mixes. Weed biomass increased in the clover mix but decreased in the native grass mix. Grapevine petiole nitrogen content was highest in the bell bean mix and lowest in the native grass mix. There were few differences in yields or juice Brix, pH, or titratable acidity in any year. However, informal tasting of small wine lots found the disked control was least favored by the 11 tasters. Cover cropped soils had greater microbial biomass than disked or berm soils, and the no-till mixes had greater microbial biomass than the disked mixes. Gophers were numerous in 1999 only, with nearly all activity exclusively in the clover mix.

* indicates corresponding author

Effect of Fertilizer Application Levels on Anthocyanoplast Development in Pione Grape Skin under a Root-Zone Restricted Condition

Goro Okamoto,* Hironori Onishi, and Ken Hirano

Faculty of Agriculture, Okayama University
Tsushima-Naka, Okayama 700-8530, Japan
[Fax: 81-86-251-8388; email: gokamoto@cc.okayama-u.ac.jp]

Effects of high fertilizer application on berry ripening and anthocyanoplast (ACP) development in skin of Pione grapes (tetraploid) were investigated. Four-year-old vines, grafted on SO4 rootstocks, were planted in root-zone restricted beds and complete liquid fertilizers containing 60 mg/L (normal; N), 90 mg/L (high; H), and 120 mg/L (excessively high; EH) of nitrogen were applied. The fertilizer level of each treatment was decreased to one-third after veraison. Juice TSS content was lower in EH-treated vines at veraison, although no significant difference was found among the three treatments at the premature (13 days after veraison) and full ripe (31 days after veraison) stages. Skin anthocyanin contents in N- and H-treated vines increased at a constant rate after veraison, while the content in EH vines was significantly lower. ACPs were first observed in epidermal and outermost hypodermal cells at veraison in N and H vines and 12 days later in EH vines. The number of epidermal cells containing ACPs increased thereafter in each treatment, but the number of hypodermal cells with ACPs increased only in N vines. The average numbers of ACPs per epidermal and hypodermal cell were largest in N vines, followed by H and EH vines when counted at the premature stage. The number of ACPs decreased thereafter because most ACPs coalesced. Maximum diameters of ACPs ranged mainly between 15 and 35 μm in N vines at the full ripe stage, whereas those in H and EH vines were less than 10 μm. From these results, the excessively high level of fertilizing inhibits ACP formation both in epidermal and hypodermal cells at veraison and in their coalescence thereafter, resulting in poor coloration of Pione grape berries.

Bird Foraging Behavior in Vineyards

Valerie Saxton,* Glen Creasy, Adrian Paterson, Michael C.T. Trought, and Graham Hickling

Soil, Plant and Ecological Sciences Division, Lincoln University
PO Box 84, Canterbury, New Zealand
[Fax : 64-3-3253843; email: saxtonv@lincoln.ac.nz]

Blackbirds (*Turdus merula*) and silvereyes (*Zosterops lateralis*) are serious vineyard pests in Australia and New Zealand. An experimental method using time-lapse video was developed to study in detail foraging decisions of these species in vineyards and in an experimental situation with a feeder table. On the feeder table, birds were offered an artificial grape where confounding parameters associated with ripening grapes, such as increasing sugars, color, and phenolic compounds, and decreasing acids, could be separated. Behavioral responses of the birds were similar in both situations, demonstrating that the experimental situation closely reflected the natural. Blackbirds returned regularly, were solitary, took whole grapes, spent a short time only on the grapes, and preferred a high (up to 40%) sugar concentration, while silvereyes clustered, visited in small flocks, pecked at many grapes, spent a longer time on the grapes than did blackbirds, and preferred a sugar concentration of 10 to 15%. Glucose absorption mechanisms of protein-eating birds have been shown to be different from those of many honey-eating birds. The type of damage inflicted on grapes by these two species, their behavior in the vines, and their social and cultural interactions are consistent with this explanation, so it may be that a physiological difference underpins the basic ecology of the two species. Knowledge of factors governing bird decision-making mechanisms may help in modeling bird behaviors and populations, and thus may lead to more successful management of birds in vineyards.

*** indicates corresponding author**

Canopy Developmental Stages for Cordon-Trained Spur-Pruned Grapevines

Michael J. Sipiora* and J.R. Lissarrague

Departamento de Producción Vegetal, Fitotecnia, Escuela Técnica Superior de Ingenieros Agrónomos de Madrid, Universidad Politécnica de Madrid, Spain
Present address: Huneeus Vintners, 1040 Main St. Suite 204, Napa, CA 94559
[Fax: 707-963-7267; email: michael@quintessa.com]

Canopy development of cordon-trained spur-pruned grapevines grown in a moderate Mediterranean climate occurs in four stages. The first stage begins at budbreak; the second begins with lateral growth initiation and ends at fruit set; the third covers the period between fruit set and pea size; and the fourth lasts from pea-sized fruit to harvest. Average temperature and number of growing degree days increase in each stage. The developmental rates of biomass accumulation and leaf area for the major canopy components (main shoots, lateral shoots, and clusters) were followed in Tempranillo grapevines with a shoot density of 8 and 14 shoots per meter, cultivated in nonirrigated and deficit-irrigated conditions. Main shoot vigor was highest during the second stage. Lateral shoot vigor never reached levels of main shoot vigor, yet lateral shoot development occurs over a longer period. Main shoot leaf area development occurs primarily before fruit set, while lateral shoot leaf area development occurs during stages two and three. Dry matter accumulation rates for clusters were highest during the last stage, which is also the longest. Dry matter accumulation rates were highest for clusters at the 14-shoot density and highest for main shoots at the 8-shoot density. Shoot-density treatment influences on canopy developmental stages were observed primarily during the first and third stages. Depending upon season, irrigation had no or little impact on canopy development in this study, as irrigation was not initiated until the third stage.

Metabolomic Profiling of Wine and Must

Rebekah Woolsey, Erika Nedeau,* David Quilici, Grant R. Cramer, John C. Cushman, and David Schooley

Department of Biochemistry, University of Nevada, Reno, NV 89557
[Email: erikan79@hotmailcom]

Wine flavor and aroma are dependent on volatile compounds derived from phenolics and primary metabolites. Water deficiency affects these metabolites, and it has been hypothesized that drought-stressed winegrapes contain more concentrated compounds responsible for the characteristic flavor and aroma of that winegrape varietal. Preliminary studies using solid-phase microextraction and GC analysis suggest significant qualitative and quantitative differences in the GC profiles of wine must produced from well-watered and drought-stressed grapes. Further analysis will be done using mass spectrometry.

*** indicates corresponding author**

Evaluation of a Sequential Ammonia and L-Arginine Enzymatic Analysis Method for Yeast Assimilable Nitrogen

Geoffrey Anderson*

Unitech Scientific, 19912 Corby Ave., Lakewood, CA 90715
[Fax: 562-809-3140; email: geoff@unitechscientific.com]

A quantitative, enzymatic end-point method for the sequential spectrophotometric determination of ammonia and L-arginine, based on established reaction sequences catalyzed by glucose dehydrogenase, L-arginase, and urease, was developed into a commercial reagent kit. The refrigerated reagent shelf life was estimated by accelerated stress analysis to be 18 months. Assay results were linear for ammonia to 400 mg/L and for L-arginine to 500 mg/L. Grape must samples were assayed without decolorization. The L-arginine recovery in aqueous standards compared with that in spiked must samples demonstrated a negative interference arginine recovery in the presence of high ammonia (>200 mg/L) and endogenous compounds in must, presumably elevated amino acid content. Nitrogen from L-arginine by this method was compared with nitrogen by the nitrogen by *o*-phthaldialdehyde (NOPA) method in 15 early and late fermentation samples: correlatation coefficient was 0.932, slope was 1.9, and intercept was 56 mg nitrogen/L; the L-arginine nitrogen ranged from 0 to 159 (mean = 50), and total primary amino nitrogen by NOPA ranged from 22 to 425 (mean = 153) mg nitrogen/L. Results of this evaluation indicate that L-arginine comprises about one-third of the primary amino nitrogen content of grape must and that determination of total yeast assimilable nitrogen may be conveniently and reliably estimated using this sequential enzymatic ammonia and L-arginine reagent.

Comparison of Media to Isolate *Brettanomyces bruxellensis* from Wine

Lorenza Conterno,* Benjamin R. Capraro, and Thomas Henick-Kling

Cornell University, Department of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, NY 14456
[Fax: 315-787-2284; email: lc@nysaes.cornell.edu]

Brettanomyces is a common contaminant that can produce potent off-flavors that lower the quality of wines. There is strong interest in the study of *Dekkera/Brettanomyces* yeasts to increase knowledge of their biology and to prevent wine spoilage. The selective detection and recovery of *Brettanomyces* yeast from materials that contain a variety of other yeasts is needed. At times these yeast are viable in wine but will not grow on culture media. Specific detection will be a powerful tool to help prevent unwanted spread of *Brettanomyces* in wines. For that purpose, several semiselective media have been developed. In addition, bacteriological media are effective in the differentiation of *Dekkera*. However, there has been no direct comparison of media to determine which are more effective in recovering *Dekkera/Brettanomyces* sp. Our aim was to compare yeast mold agar with cycloheximide (YMC) to five other media formulated for *Brettanomyces* detection. The plates were inoculated with liquid pure *Brettanomyces* culture, one month old. YM agar was used as reference for the total recoverable colony forming units. All media showed a high decrease in culturability, as compared to YM agar. The medium with a combination of ethanol and cycloheximide suppressed the growth of all cells. Among the media tested, YMC showed the best compromise between selectivity and culturability.

* indicates corresponding author

Measurement of Key Fermentation Parameters of Strains of Wine Yeast by FTIR Spectroscopy during Fermentation of Cabernet Sauvignon and Chardonnay Juices

Jeffrey Farthing, Kenneth Fugelsang, and Roy Thornton*

Department of Viticulture and Enology, California State University, Fresno
2460 E. Barstow Ave., Fresno, CA 93740
[Fax: 559-278-4795; email: rthornto@csufresno.edu]

Multiparametric measurements of fermentation have been time-consuming and retroactive. Fourier transform infrared (FTIR) spectroscopy offers real-time measurements during fermentation and the potential to identify problems in fermentation as they occur. Wine yeast strains differ in their ability to ferment wine grape juices. In this study, the ability of five yeast strains (Premier Cuvee, Cote des Blancs, Ruby.ferm, Noble.ferm, and Zymaflore VL1) to ferment Cabernet Sauvignon was evaluated. Five yeast strains (Premier Cuvee, Cote des Blancs, Zymaflore ST, ICV-GRE, and SM102) were evaluated in Chardonnay juice. The yeasts were inoculated in triplicate into both juices. The fermentation temperature was maintained at 18°C for Chardonnay fermentations and 23°C for Cabernet Sauvignon fermentations. Samples were taken daily and analyzed by FTIR spectroscopy. Premier Cuvee and Cote des Blancs completed fermentation of the Cabernet Sauvignon juice in 14 days, compared to 16 days for Ruby.ferm, 27 days for Zymaflore VL1, and 34 days for Noble.ferm. Ethanol production ranged from 14.5 to 15% v/v, and volatile acidity ranged from 0.18 to 0.48. With the exception of Cote des Blancs, the initial fermentation rates in Chardonnay juice for the yeast strains were similar. The strains differed in their ability to complete fermentation to dryness. Premier Cuvee completed fermentation in 22 days, followed by 28 days for ICV-GRE and 35 days for Zymaflore ST. The Cote des Blancs and SM102 fermentations had >1% sugar after 43 days. Cote des Blancs produced more volatile acidity than the other yeast strains.

Effect of Overexpression of *MET17* Gene on Hydrogen Sulfide Formation in Twelve Wine Yeasts

Yeun Hong and Linda F. Bisson*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email: lfbisson@ucdavis.edu]

Control of hydrogen sulfide (H_2S) production by yeasts is important in wine fermentations because of its negative aroma and low sensory threshold. Yeasts produce H_2S from various sulfur sources such as sulfate, amino acids, and glutathione. Nitrogen deficiency in grape must has also been shown to induce H_2S production. *MET17* encodes for *o*-acetylhomoserine/*o*-acetylsulfhydrylase that incorporates sulfide (S^{2-}) into carbon chains. It is postulated that the overexpression of the *MET17* gene can increase the activity of this enzyme and boost reduced sulfur incorporation, causing H_2S production to decrease. To investigate the effect of overexpression of *MET17*, 12 yeasts were transformed with a plasmid containing the *MET17* gene and with the vector as a control. Fermentations were conducted in a synthetic juice medium,Triple M, with two different nitrogen concentrations of 433 and 208 mg/L N equivalents. To estimate the loss of CO_2, flask weights were monitored every 24 hours. H_2S detecting tubes were used to measure the amounts of H_2S produced. All fermentations were performed in duplicate. Control and transformed strains showed 81 to 97% theoretical carbon loss. Four of 12 yeasts transformed with *MET17* showed a lower H_2S production than the control transformants. The other strains showed no change in the level of H_2S. All yeasts produced more H_2S with low nitrogen. The transformants were also evaluated in grape juice. These results suggest that some strains may be deficient in reduced sulfur incorporation, but other strains showed no effect. Multiple genetic factors likely contribute to the sulfide-producing behavior of native yeast strains.

* indicates corresponding author

Diversity of *Brettanomyces/Dekkera* Isolated from Wine

C.M. Lucy Joseph* and Linda Bisson

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email: cmjoseph@ucdavis.edu]

Brettanomyces/Dekkera strains from the Wine Yeast and Bacteria Collection at UC Davis and the collection at Cornell University were examined for physiological and metabolic diversity under laboratory conditions. From an initial list of 49 isolates, 36 were chosen for further analysis based upon the type of wine and geographic area in which they were isolated. Strains were tested for growth on various carbon sources, nitrogen sources, vitamins, and under different environmental conditions. Results indicate that there are some common characteristics among the *Brettanomyces* strains but that they are highly variable in other characteristics. Most of the strains have an absolute requirement for the vitamins biotin and thiamin but do not require any amino acids for growth. All grew well on glucose, fructose, and sucrose, and most grew well on galactose, maltose, and trehalose. No isolates tested show growth on cellulose alone as a carbon source. The ability to use other carbon sources was variable. *Brettanomyces* strains differed widely in their tolerance to high temperature and alcohol when grown in minimal media. A few of the isolates appear to be identical or extremely similar based on preliminary results. Using the classic taxonomic scheme for classifying *Brettanomyces/Dekkera* (*The Yeasts, A Taxonomic Study*, C.P. Kurtzman and J.W. Fell, eds.), 8 of these strains are *Dekkera anomala*, 17 *Brettanomyces naardenensis*, 8 either *D. bruxellensis* or *B. naardenensis*, and 3 not classified.

Effect of Yeast Inoculation Rate and Acclimatization to Juice on Icewine Fermentation

Derek Kontkanen and Debra Inglis*

Cool Climate Oenology and Viticulture Institute, Brock University
500 Glenridge Ave., St. Catharines, Ontario, Canada L2S 3A1
[Fax: 905-688-3104; email: dinglis@spartan.ac.brocku.ca]

Icewine is an intensely sweet, unique dessert wine fermented from the juice of grapes that have frozen naturally on the vine. The juice pressed from the frozen grapes is highly concentrated, ranging from a minimum of 35 to -42 Brix. Icewine fermentations are often sluggish, taking months to reach the desired ethanol level, and sometimes become stuck. In addition, icewines have high levels of volatile acidity. At present, there is no routine method of yeast inoculation for fermenting icewine. This project investigated two yeast inoculum levels, 20 and 50 g/hL, and compared the fermentation kinetics of inoculating these levels directly into the icewine juice or conditioning these cells to the high sugar levels using a stepwise acclimatization procedure. The effect of adding a yeast nutrient was also assessed. Yeast inoculated at 20 g/hL stopped fermenting before the required ethanol level was achieved, producing only 7.8 and 8.1% (v/v) ethanol for the direct and conditioned inoculations, respectively. At 50 g/hL, the stepwise conditioned cells fermented the most sugar, producing 12.2% (v/v) ethanol, whereas the direct inoculum produced 10.5% (v/v) ethanol. The addition of the yeast nutrient increased the rate of biomass accumulation, but reduced the ethanol concentration in wines fermented at 50 g/hL. There was no significant difference in acetic acid concentration in the final wines across all treatments.

* indicates corresponding author

Analysis of Low Hydrogen Sulfide Production by *Saccharomyces cerevisiae* Strain UCD932

Angela Lee and Linda Bisson*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Tel: 530-752-0382; email: lfbisson@ucdavis.edu]

Production of hydrogen sulfide (H_2S) during alcoholic fermentation by *Saccharomyces cerevisiae* is a problem in the wine industry because it leaves the end product with a rotten-egg characteristic. Environmental and nutritional factors have been associated with the production of volatile sulfur compounds but production levels vary across strains in response to these conditions. This variation has been attributed to the ability to incorporate reduced sulfur into organic compounds and suggests that differences in internal enzyme regulation and activity affect H_2S production. One strain of *Saccharomyces cerevisiae*, UCD932, is an extremely low producer of H_2S under all conditions investigated and we hypothesized that it carries alleles that reduce production of H_2S. Sequence analysis of several genes (*MET17, CYS4*, and *MET6*) known to be responsible for variable levels of H_2S production in brewing yeast strains was performed in native and commercial isolates. UCD932 carried non-neutral mutations in both *CYS4* and *MET6*. The *CYS4* and *MET6* allele variants were obtained from UCD932 and used to transform high H_2S-producing strains to determine if the presence of either allele alone was sufficient to reduce sulfide formation. The specific allele of *CYS4* present in a strain (*CYS4*^{WT} or *CYS4*^{UCD932}) did not affect the sulfide production behavior of the strains. The *MET6* experiments are still in progress as multiple strains contained varying alleles of this gene.

Effects of Yeast Preadaptation on Low-Temperature Wine Fermentation

Josep M. Llauradó,* Nicolas Rozès, Magdalena Constantí, and Albert Mas

UE-CeRTA. Dept. Bioquímica i Biotecnologia, Fac. Enologia
Universitat Rovira i Virgili, Ramon i Cajal, 70, 43005 Tarragona, Spain
[Fax: 34 977 25 0347; email: jmlr@astor.urv.es]

Low-temperature fermentations (10 to 15°C) are becoming increasingly interesting for producing wines with high aromatic profiles. Low temperatures, however, can restrict the normal development of yeast and increase the risk of stuck and sluggish fermentations. We analyzed how, at this limiting temperature, previous subcultures of wine yeast affected fermentation. Three types of wine yeast were used, all identified as *Saccharomyces cerevisiae* and commercially identified as p.r. *cerevisiae* (strain 1), p.r. *bayanus* (strain 2), and p.r. *uvarum* (strain 3, considered to be "cryotolerant"). Strains 1 and 2 were used in three ways: rehydrated (control), rehydrated and subcultured three times at 13°C (cold preadapted), and rehydrated and subcultured three times at 25°C (fermentation starter). Strain 3 was used only as in the control condition. In all cases, microvinifications (700 mL) were carried out at 13°C and inoculated with 2 x 10^6 cells/mL on fresh Cariñena grape musts. Amino acids and ammonium consumption were monitored during fermentation. For strain 1 there were no differences between the three conditions. When strain 2 was cold preadapted, the rates of fermentation were faster and the length of fermentation was shorter. Strain 3 displayed a sluggish fermentation. The consumption of nitrogen was more rapid when the fermentations were faster. The strain of yeast is the most important variable for achieving an acceptable fermentation performance at low temperatures, although in some cases performance may be improved by preadaptation.

* indicates corresponding author

Macerating Enzymes and Grape Seed Tannin for Color Extraction and Retention in Cynthiana Wine

Gary L. Main* and Justin R. Morris

University of Arkansas, Institute of Food Science and Engineering
Fayetteville, AR 72704
[Fax: 479-575-2165; email: gmain@uark.edu].

Cynthiana, *Vitis aestivalis*, a red winegrape, was fermented using macerating enzymes and grape seed tannin to evaluate their effect on color extraction, phenolics, and color retention. In experiment 1, the absorbance spectrum from 220 to 460 nm was measured for six days during fermentation on the skins. Five commercial macerating enzymes, Trenolin color DF, Lallzyme EX-V, Crystalzyme Tinto, Rohapect VR-C, and Vinozyme G, and no enzyme were tested at the maximum manufacturers recommended usage level. Maximum red color, absorbance at 530 nm, was achieved on day 3 of fermentation and then declined. The highest phenolic levels, absorbance at 280 and 330 nm, were recorded on day 5 of fermentation and then declined. Absorbance spectra were similar on day 8 among all treatments after pressing day 7. Experiment 2 was established by combining three replicates within each enzyme treatment from experiment 1. This wine was divided into two replications with and without grape seed tannin [Grap'tan PC (20 g/HL)]. Tannin was applied before malolactic fermentation, after which pH was adjusted to 3.55 with tartaric acid and wines were cold stabilized before bottling. In the newly bottled wine, there were no differences in the absorbance at 280, 330, or 420 nm due to enzyme or tannin treatment. Absorbance was higher at 530 nm in wines treated with Vinozyme G than in the no enzyme treatment wines. No effect on red color due to tannin addition was seen in the newly bottled wine.

Persistence of Non-*Saccharomyces* Yeast Nucleic Acids in Wine Fermentations

Trevor G. Phister, Luca Cocolin, and David A. Mills*

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email: damills@ucdavis.edu]

A number of molecular biology techniques such as PCR and PCR-denaturing gradient gel electrophoresis (DGGE) have been developed to directly detect yeasts and bacteria in wine fermentations. However, a positive PCR result can be difficult to interpret as the relationship between the persistence of nucleic acids and the persistence of metabolically active cells remains to be determined. Using specific PCR and PCR-DGGE, we demonstrated that molecular signatures of *Hanseniaspora* and *Candida* can persist in wine fermentations long after viable (colony forming) cells were undetectable. To further examine the persistence of nucleic acid in fermentations, real-time PCR assays were developed to better quantify *Hanseniaspora* and *Candida* DNA. Real-time PCR assays specific for each yeast were developed such that the target population could be detected in the presence of a 10,000-fold greater concentration of *Saccharomyces* DNA. Real-time PCR values were compared to a standard curve of known concentrations of yeasts and to plating results from mixed culture fermentations. Given the increased use of direct molecular methods to enumerate microorganisms in wine, the developed correlations will help define the relationship between the persistence of nucleic acids and the metabolic state of the cognate yeast population.

*** indicates corresponding author**

Sparkling Wine Polysaccharides during Second Fermentation and Aging in Contact with Lees

M. Riu-Aumatell, M. López-Barajas,* E. López-Tamames, and S. Buxaderas

Escola de Ciències Experimentals i Tecnologia, Universitat Internacional de Catalunya Nova Estació s/n, 43500 Tortosa, Tarragona, Spain
[Tel: 34 977 50 30 36; fax: 34 977 50 43 45; email: magdalb@unica.edu]

The effect of a second fermentation and aging in contact with lees on sparkling wine polysaccharides of different molecular weight was evaluated. Four different base wines and their corresponding cavas were compared according to the content and/or type of polysaccharides during the second fermentation and 21 months of aging in contact with lees. Six fractions of polysaccharides of different molecular weight were obtained in the base wines. The same six fractions of polysaccharides were obtained in all corresponding cavas. After nine months of aging (the legal minimum period of aging for cava), the content of all the fractions decreased as compared to the base wine. After 15 months of aging in contact with lees, the polysaccharides increased greatly, reaching a maximum value. This could be due to the yeast autolysis. After ~18 months of aging, the content of the six fractions of polysaccharides decreased again, probably due to enzyme activities. Three cavas exhibited the same behavior during aging, while the fourth cava exhibited a more rapid change.

Application of Microwave Vacuum Dehydration for Processing of Grapes Suitable for Dessert Wines

Andrew X. Gamache*

Department of Biological Systems Engineering, Washington State University
PO Box 646120, Pullman, WA 99164
[Tel: 509-335-8061; fax: 509-335-2722; email: agamache@wsu.edu]

The goal of the research was to enhance the compositional characteristics of White Riesling grapes for the production of an icewine-style dessert wine. A microwave vacuum dehydration vessel was used for the partial dehydration of White Riesling grapes to increase the sugar content from 20 to 40 Brix before fermentation. Sensory and compositional analysis of the enhanced wine were made against two artificially frozen treatments, one harvested at the same time as the microwave vacuum treatment and the other after the first freeze of the harvest season. All treatments were fermented with *Saccharomyces cerevisiae* V1116 yeast.

* indicates corresponding author

Communicating Wine Sensory Benefits to Consumers Using Descriptive Analysis

Isabelle Lesschaeve*

Inno Vinum, 615 Leblanc E., Longueuil, QC J4J 1L8, Canada
[Fax: 450-674-6157; email: isabelle@innovinum.com]

Descriptions of wine sensory characteristics, generally issued by wine experts, are widely used to promote wines or guide consumer purchases. However, several studies have shown that consumers, when tasting in blind conditions, seldom recognize a wine based on its expert description. This research proposed the use of sensory descriptive analysis as a tool to link expert and consumer languages and to improve communication of wine sensory benefits. A group of Quebec oenophiles, trained in traditional wine tasting, provided a description of the appearances, aromas, and flavors of five Washington State Merlot wines. Forty-one red wine consumers from the Toronto area participated in two sessions to collect liking scores for each wine sample as well as descriptors regarding likes and dislikes. A descriptive analysis was conducted to determine the sensory attributes that differentiated each wine and relate them to both expert and consumer languages. Eight trained panelists, experienced in wine descriptive analysis, participated in six hours of training; sensory measurements were duplicated. Sensory descriptive data were correlated to both expert and consumer descriptive data. Results indicated the power of descriptive analysis to understand descriptors and translate them into expert words. Similarly, it was possible to highlight words that were more appropriate to communicate expert opinions to consumers.

Natural Seasoning Monitoring of Cooperage Oak Using Electronic Tongue Sensors

Jean-Louis Puech,* Andrei Prida, Sandrine Isz, and Christian Radoux

Institut National de la Recherche Agronomique, Unité Mixte de Recherches Science pour l'Œnologie, 2, Place Viala, 34060 Montpellier, France
[Fax: 33-04-99-61-26-83; email: puechjl@ensam.inra.fr]

This study estimated the optimal duration of seasoning by analyzing the overall taste of oak extracts. Changes in the oak during natural seasoning were monitored using an electronic tongue system. The naturally seasoned sessile oak samples corresponded to green wood and wood after 6, 12, 18, 24, and 36 months of seasoning. The wood extracts were analyzed by an array of global selectivity chemical sensors (electronic tongue). Principal component analysis was used to discriminate between different seasoning levels. Monodimensional transformation of sensor signals was used to correlate the electronic tongue response with the duration of seasoning. Total and free ellagic acid content of the same oak samples was measured by HPLC. Good discrimination between the different seasoning levels was found (discrimination index 88). A period of constant change in the chemical composition of oak was observed, followed by a period of stability after 18 months of seasoning. During the period of constant change, the electronic tongue signal closely correlated with the duration of seasoning. Changes in the amount of total and free ellagic acid are not easily interpreted as two phenomena are probably involved: ellagitannin hydrolysis and leaching by rain. Results showed that the overall taste of wood extracts, itself related to oak extractives, was correlated with the duration of seasoning. The electronic tongue approach provided a simple and rapid method of seasoning control.

* indicates corresponding author

Characterization of Chitinases from Muscat Bailey A Grape Skins

Tsutomu Takayanagi, Tohru Okuda, Nobutoshi Kiba, and Koki Yokotsuka*

The Institute of Enology and Viticulture, University of Yamanashi
1-13-1 Kitashin, Kofu, Yamanashi 400-0005, Japan
[Email: yokotsuk@ccn.yamanashi.ac.jp]

Plant chitinases are thought to be closely related to a plant's defense system against phytopathogens. The characteristics of chitinases separated from Muscat Bailey A (Bailey x Muscat Hamburg) grape skins were investigated. The fraction that exhibited chitinase activity was fractionated from the extract of the grape skins by affinity chromatography with chitin beads and then separated by cation-exchange chromatography. The cation-exchange chromatography yielded two protein peaks (CHI-A and -B) that exhibited chitinase activity. The molecular weights of CHI-A and -B were estimated to be 29 and 27 kDa, respectively, by SDS-PAGE. The partial amino acid sequences of CHI-A and -B were highly homologous with that of the class IV chitinase of *Vitis vinifera*. The optimum pH of CHI-A and -B was 5.0 and the enzymes were stable over the pH range of 3.0 to 7.0. The optimum temperature of CHI-A (70°C) was higher than that of CHI-B (50°C). CHI-A and -B inhibited the growth of *Botrytis cinerea* in an in vitro test using liquid culture.

Treating *Brettanomyces* in Oak Cubes with Gaseous and Aqueous Ozone

Nicolas O. Cantacuzene, Erin S. Dormedy,* Joseph L. Smilanick, Kenneth C. Fugelsang, Robert L. Wample, Denis J. Bacon, and Derek F. Dormedy

California State University, Fresno, 5300 North Campus Drive, Fresno, CA 93740 [Fax: 559-278-7623; email: edormedy@csufresno.edu]

The efficacy of ozone as a sanitizer to eliminate *Brettanomyces bruxellensis* found in barrels was evaluated by treating colonized oak cubes of 1 cm^3. Oak cubes were used to overcome the difficulty of accurately determining the population of *Brettanomyces* found in barrels. An initial wine broth was spiked with a culture of *B. bruxellensis*, and oak cubes were introduced. After a haze had formed in the wine broth, a sample of oak cubes was ground in 100 mL of sterile water, and a 0.2-mL aliquot was retrieved to estimate the population of *Brettanomyces* that had colonized the wood. Gaseous ozone treatment consisted of placing a random sample of oak cubes into an ozone gas chamber. Gaseous ozone was applied at 600 and 1300 mg/L at times of 15 min, 30 min, 1 hr, and 2 hr. The second treatment consisted of applying different concentrations of ozonated water of 1, 3, and 5 mg/L to a random sample of cubes for a period of 3 min. The third treatment consisted of placing a random selection of oak cubes in hot water at 82°C for a period of 20 min. Results showed that ozone gas had an effect on the *Brettanomyces* population, whereas the aqueous ozone had no effect on *B. bruxellensis* contained in oak cubes. Like gaseous ozone, hot water had an effect on the *B. bruxellensis* population found in oak cubes. In addition, a scanning electron microscope was used to determine the efficacy of ozone 2 mm inside the oak cubes.

*** indicates corresponding author**

Impact of Soil Type on Survival of Grape Phylloxera in the Pacific Northwest

Rebecca Chitkowski and James R. Fisher*

U.S. Department of Agriculture-Agriculture Research Service, Horticulture Crops Research Laboratory, 3420 NW Orchard Blvd., Corvallis, OR 97330
[Tel: 541-738-4032; email: fisherj@science.oregonstate.edu]

The survival of grape phylloxera in vineyards is highly dependent on abiotic environmental factors. It has been suggested that soil type plays an integral part in phylloxera survival and dispersal. The purpose of this experiment was to determine whether phylloxera are able to survive and reproduce in different soil types from the Pacific Northwest. Samples for this study included two representative soils from the Willamette Valley, OR, that are known to support phylloxera populations (Jory and Helvetia) and a soil from the Rogue Valley (Abegg). We also included a soil from the Yakima Valley, WA (Burke), and two artificial soils, a soilless mix and a Jory-sand mix. *Vitis vinifera* var. Pinot noir cuttings were planted in 10.2-cm pots of each soil. Established plants were infested twice with phylloxera eggs originating from Washington and Oregon. Treatments of phylloxera from each origin and in each soil type were replicated 15 times. After two months, phylloxera numbers were collected through investigation of core soil samples. All six soil types supported phylloxera infestations, regardless of population origin. However, soil from the Yakima Valley had the lowest phylloxera population numbers, whereas soils from the Willamette Valley, Rogue Valley, and the artificial mixes consistently had a high survival of phylloxera.

Developing Gene Expression Profiles for Research on Grape Responses to *Xylella fastidiosa*

Hong Lin* and M. Andrew Walker

U.S. Department of Agriculture-Agriculture Research Service
9611 South Riverbend Ave., Parlier, CA 93648
[Fax: 559-596-2921; email: hlin@fresno.ars.usda.gov]

Pierce's disease (PD), caused by the bacterium *Xylella fastidiosa (*Xf), is one of the most devastating grape diseases. However, information regarding the molecular basis of the host plant response to Xf infection is limited. In order to characterize the molecular events in the grape Xf interaction, we are developing a genomic approach to identify transcriptional pathways correlated with susceptibility and resistance. Highly resistant and susceptible genotypes were selected from a *Vitis rupestris* x *Muscadinia rotundifolia*. RNA samples will be collected over time intervals from each genotype, and infected and control plants will be used to construct subtractive cDNA libraries that will represent a complete expression profile of each genotype in response to Xf. This subtractive technique for cDNA library construction will help remove the most common housekeeping genes and maximize identification of genes that specifically respond to Xf. About 6,000 to 8,000 clones will be sequenced from each library. These sequences will be put into a PD expression profile database and homology-based comparisons will be made among published grape, Arabidopsis, and other public plant databases. A subset of candidate genes will be selected for differential expression analysis using microarrays. The results derived from this study will help reveal the molecular mechanism(s) of grape resistance and susceptibility to Xf.

* indicates corresponding author

Progress toward Mapping Root-knot Nematode Resistance Derived from Grape Rootstock Ramsey

Kristin M. Lowe and M. Andrew Walker*

Department of Viticulture and Enology, University of California
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email:awalker@ucdavis.edu]

Root-knot nematodes (RKN) (*Meloidogyne* spp.) are serious pathogens of grapevines worldwide, reducing vine growth and productivity. Traditional control methods are limited or economically unviable for viticulture, dictating a need for broad-based, durable host-plant resistance. To facilitate rootstock breeding efforts, we are currently researching the genetics of resistance to RKN, with the ultimate goal of identifying the resistance gene. Previous studies on the inheritance of RKN resistance in grape species indicated that resistance in *Vitis champinii* is conferred through a single, dominant gene. An F1 population derived from an interspecific rootstock cross of Ramsey (*V. champinii*) x Riparia Gloire (*V. riparia*) (PC9715) segregates 1:1 for this trait, suggesting parental genotypes of Nn and nn (heterozygous resistant x homozygous susceptible). Resistance for this population was evaluated by counting egg mass number on 14-week-old inoculated seedlings and those with ≤2 egg masses per root mass were defined as resistant. We are now generating a map for this population following a pseudotestcross strategy using amplified fragment length polymorphism (AFLP) and microsatellite markers, with a goal of 500 total markers. A bulk segregant analysis study is underway to find specific molecular markers segregating with the resistance gene in the population. The mapping population consists of 250 individuals, and preliminary molecular marker screens have shown significant polymorphism between resistant and susceptible individuals.

Powdery Mildew Management in Susceptible Vineyards in California

George Leavitt* and **Tomé Martin-Duvall**

University of California Cooperative Extension, 328 Madera Ave., Madera, CA 93637
[Email: gmleavitt@ucdavis.edu]

Powdery mildew (*Uncinula necator*) is the most serious disease of grapes (*Vitis vinifera*) in California. Powdery mildew can reduce berry size, reduce storage life of table grapes, and promote bunch rots by causing the berries to crack. The ultimate result is reduction in yield and possible rejection of the crop at the winery or packing shed. Under very favorable conditions in susceptible varieties, mildew can cycle about every seven days. With this short generation interval comes an increased tendency toward resistance buildup. Consequently, there is a need for rotation of chemistries, new chemistries, and acceptable resistant varieties. Utilizing the mildew model can reduce the number of applications needed in a year, reducing the potential for resistance and disease-management related costs. Studies were conducted in a very susceptible Carignane vineyard in two subsequent years, one with relatively heavy and the other with relatively light mildew pressure. There are promising new materials moving through the registration process and new methods of using older materials, including stretching or shortening application intervals based on the University of California Powdery Mildew Model.

*** indicates corresponding author**

Berry Protein Identification from Drought-Stressed and Well-Watered *Vitis vinifera* Vines

Rebekah Woolsey,* David Quilici, Kathleen Schegg, Alaine Garrett, David Schooley, John C. Cushman, and Grant R. Cramer

Department of Biochemistry, University of Nevada, Reno, NV 89557
[Email: wwoolsey@cccomm.net]

Winegrapes are being considered as an alternative agricultural crop to alfalfa for northern Nevada, particularly because of the crop's low water use compared to alfalfa. Water deficiency can alter fruit composition and influence wine color, taste, and aroma. To better understand these metabolic changes, we examined changes in protein profiles between well-watered and drought-stressed grapes. We have developed protein extraction and identification protocols. Proteins were extracted by using sequential extraction buffers (BioRad). After extraction, the proteins were separated by isoelectric point and molecular weight through two-dimensional gel electrophoresis. Spots were analyzed using PDQuest software, followed by characterization of both soluble and membrane bound proteins by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF)-TOF (ABI 4700 proteomics analyzer). Protein profiles for well-watered and regulated deficit irrigated grapes were examined, and proteins differing in the two profiles were identified.

Integrated Disease and Insect Management Programs for Norton Grapevines in the Midwest

Imed Dami,* Kaan Kurtural, Mohammad Babadoost, and Richard Weinzierl

Department of Plant Soil and General Agriculture, MC 4415, 1205 Lincoln Dr., Southern Illinois University, Carbondale, IL 62901 [Fax: 618-453-7457; email: imeddami@siu.edu]

An integrated disease and insect management program for Norton was developed in order to reduce the amount and frequency of applied chemicals. Norton is a promising winegrape cultivar in the Midwest with moderate disease resistance. Specific objectives were to evaluate the effectiveness of integrated disease and insect management programs as compared to standard programs currently used by grape producers in Illinois and to identify the most economical and sustainable disease and insect management programs. The spray program included three treatments: standard spray program (STD) (10 to 12 sprays per season) normally used by grape producers; integrated pest management (IPM) program or reduced spray program (4 to 6 sprays per season); and control with no spray. The control treatment showed the highest disease and insect pressures; the STD program showed the lowest; however, the IPM program was similar to the STD program. Control clusters had higher bunch rot incidence than those of the STD and IPM programs. Yield components were not affected by treatments. Fruit composition (soluble solids, pH, and titratable acidity) was not affected by treatments when clusters were culled after harvest. The IPM program has provided sufficient disease and insect control similar to that of the STD program; but the cost of pesticides per acre for IPM was cut by an average of 60% over a 3-year period. The IPM program was less costly, thus more profitable, and less harmful to the environment than the STD program, and it can thus be recommended to Norton grape producers.

* indicates corresponding author

Survival of Twelve *Vitis vinifera* Varieties for Six Years in Reno, Nevada

Jason Evans,* Elizabeth A.R. Tattersall, Wayne Johnson, and Grant R. Cramer

Department of Biochemistry, University of Nevada, Reno
Mail Stop 200, Reno, NV 89557
[Fax: 775-784-1650; email: Delekon@yahoo.com]

A one-acre experimental vineyard has been established by the College of Agriculture, Biotechnology, and Natural Resources at the University of Nevada, Reno, to study varietal survivability and wine-quality characteristics of the northern Nevada area. This region is subject to cold in the form of spring freezes and lethal temperatures, which could be a deciding factor in the feasibility of vineyard establishment. The varietal trial was constructed using a randomized block design. Twelve varieties were tested in six blocks, each containing 15 vines. Irrigation of each block was independently controlled using a drip system. Statistical analysis showed significant differences in survival rate among varieties. The effect of seasonal lows and spring freezes on survival was assessed.

Foliar Applications of Messenger Do Not Affect Physiology and Performance in Cabernet Sauvignon Grapevines

Sanliang Gu,* Robert Cochran, Kenneth Fugelsang, Robin Ross, Marie Ocafrain, and Michael Kropp

Viticulture and Enology Research Center, California State University, Fresno
2360 East Barstow Ave., Fresno, CA 93740
[Fax: 559-278-4795; email: sanliang@csufresno.edu]

Messenger, a patented product of harpin protein (EDEN Bioscience), has been demonstrated to activate systemic acquired resistance, a well-characterized plant defense mechanism. Harpin protein is effective in eliciting disease resistance and accelerating plant development in various crops including grapevines. This study investigated the effect of Messenger on vine physiology, vine performance, and fruit composition under field conditions. The experiments were designed as randomized complete blocks of four or five replications. Messenger was foliar applied to the vine canopy at 4.5 oz/A three, four, and six times between fruit set and veraison in 2002 in Lodi, Fresno, and Paso Robles (CA), respectively. Physiological measurements included stomatal conductance, transpiration, photosynthesis, quantum yield efficiency of photosystem II photochemistry, nonphotochemical quenching, and electron transfer rate of mature leaves. Berries were sampled for analysis of Brix, TA, pH, anthocyanin content, and berry weight. Weight and number of clusters were determined for yield components and yield. Petioles were sampled at veraison for mineral nutrition. Pruning weight was collected during the dormant season to evaluate vine vigor. Foliar application of Messenger in addition to the grower's standard spray program did not affect any variables measured in all locations with the exception of petiole S at veraison in Fresno, which was only 18% higher on Messenger-treated vines. Messenger can be applied to field-grown grapevines for disease control with little concern for altering vine growth, petiole mineral nutrition, vine performance, and fruit composition.

* indicates corresponding author

Mycorrhizal Fungi in Grape Roots (*var*. Blanc Du Bois): Evaluation within a Gulf Coast Vineyard

Lisa Morano*

Department of Natural Sciences, University of Houston, Downtown
One Main Street, Suite 840-N, Houston, TX 77002
[Tel: 713-221-2782; email: moranol@uhd.edu]

Mycorrhizae are mututalisms between fungal species and plant roots. Plants deliver carbohydrates to the fungus and the fungus brings nutrients from beyond the root zone directly to root cortical cells. Grapevines are typically infected with vesicular arbuscular (VA) mycorrhizae that form characteristic arbuscules, treelike structures for nutrient exchange and vesicles, spherical fungal structures probably involved in lipid storage. Rootlets (less than 1-mm thick) were collected, cleared, and stained from a total of 27 Blanc Du Bois vines within a Gulf Coast vineyard. Thirty rootlets from each vine were evaluated for the presence of fungal hyphae, arbuscules, and vesicles per 1-mm root length. For those roots with vesicles, it was noted whether there were few (1 to 2 vesicles) or many (>3 vesicles). Mycorrhizal fungi changed from 41% hyphal infection in new spring roots (April) to 85% hyphal infection in winter roots (February). Spring roots showed large variability in percent hyphae and vesicles and only a few arbuscules were identified. In winter roots there were no arbuscules, but there was an inverse correlation between cluster weight and the percent root sections with 3 or greater vesicles (r^2 = 0.40). As roots age they clearly appear to become more infected with mycorrhizae, and it also appears that the vines with smaller clusters and lower yield have greater vesicles by winter. There is no correlation of mycorrhizal infection level with soil depth, but there is a slight positive correlation of roots with 3 or greater vesicles and petiole phosphate levels (r^2 = 0.10).

Characterization of Cabernet Sauvignon Clones by Phenotype and DNA Polymorphism Analysis

Tohru Okuda, Tsutomu Takayanagi, Hiroshi Saito, Noboru Ueno, and Koki Yokotsuka*

The Institute of Enology and Viticulture, University of Yamanashi
13-1 Kitashin-1-chome, Kofu, Yamanashi 400-0005, Japan
[Fax: 81-55-220-8768; email: yokotsuk@ccn.yamanashi.ac.jp]

Seven Cabernet Sauvignon clones (A to G) were analyzed phenotypically and genetically. No difference in phenotypes was detected among their berries during maturation except that the berry weight of clone B was significantly lower than that of the other clones. DNA polymorphism of these clones was then analyzed by random amplified polymorphic DNA (RAPD), PCR-restriction fragment length polymorphism, and single-strand conformation polymorphism methods with 80 types of random 10-mer primers. Of the primers used, OPC-5 showed a band (250 bp) specific for clone F by RAPD. The band was recovered from an agarose gel and cloned, and its genetic sequence was determined. A specific primer set of this sequence was designed and sequence tagged site PCR was carried out. One strong band was detected only in one lane for clone F. Results suggested that although genetic differences among grape clones are very small, DNA analysis is a rapid and reproducible tool for distinguishing one clone (sport) from another without effects from environmental factors such as climate and viticultural procedure.

* indicates corresponding author

Vineyard Nitrogen and Water Dynamics in Perennial Clover and Bunch Grass Cover Crop Systems

Amy E. Patrick and Alison M. Berry*

Department of Environmental Horticulture, Graduate Group in Ecology,
University of California, Davis, CA 95616
[Tel: (530) 752-7683; email: amberry@ucdavis.edu]

A perennial strawberry clover cover crop and a perennial native bunch grass cover crop in a California Central Valley vineyard were analyzed for differences in water and nitrogen status. Grape leaves and soil were sampled for two growing seasons and analyzed for total nitrogen content and the natural abundance of ^{15}N. Soil of the rhizomatous clover cover crop system had high water content, total N, soluble N, and vegetative cover and low weed density. Soil in the bunch grass cover crop system had low water content, total N, soluble N, and vegetative cover and high weed density. Grape leaves in the clover system had high water content, percent N, and $\delta\ ^{15}N$. Increased $\delta\ ^{15}N$ in the clover system may be due to increased rates of anaerobic denitrification and other fractionating processes associated with N turnover in the wetter, more N-rich clover system.

Effects of Leaf to Fruit Ratio on Berry Ripening of Cabernet Sauvignon Grape Cultivated in Ningxia, China

Changbing Song,* Goro Okamoto, Ken Hirano, Haijun Ma, and Xuemei Feng

Laboratory of Viticulture and Enology, Ningxia Academy of Agriculture and Forestry Science, Xinxia Road 76, Yinchuan, Ningxia, 750021 China
[Fax: 0086-951-5049204; email: cbsong@public.yc.nx.cn]

In field trials in Ningxia, China, the effect of different leaf to fruit ratios of Cabernet Sauvignon grapevine on berry ripening was investigated. Shoots were topped to the remaining six primary leaves from the cluster, and the leaf area was arranged by eliminating the number of lateral leaves per shoot to 0 (without lateral, a traditional shoot management in Ningxia; A), 5 (with one lateral; B), and 10 (with two laterals; C). Composition of juice and pigments and phenolic substance of skin were analyzed. The leaf to fruit ratio in treatments A, B, and C was 5.26, 6.83, and 8.11 cm2/g·FW, respectively. The different effect on berry ripening was remarkable, resulting in changes of fruit composition in fruit ripening at the harvest. Berry quality was significantly improved by the treatment with two laterals, although juice composition was somewhat insufficient for quality wine, because of the lower acidity. Berry ripening was delayed in the Ningxia vineyard due to the insufficient leaf to fruit ratio. Increasing leaf numbers per shoot by no pinching and decreasing cluster number by thinning are essential for producing quality wines.

* indicates corresponding author

Yinchuan Plain: A Developing Winegrape Region in Ningxia, China

Changbing Song,* **Shengbao Li**, Haijun Ma, and Xuemei Feng

Laboratory of Viticulture and Enology, Ningxia Academy of Agriculture and Forestry Science, Xinxia Road 76, Yinchuan, Ningxia, 750021, China
[Fax: 0086-951-5049204; email: cbsong@public.yc.nx.cn]

Geography, climate, and soil were characterized in the Yinchuan Plain, a semidry region located in northwest China (long. 106', lat. 36-38'N, alt. 1050-1120 m). The growing season has 1300 to1600°C degree days, 13 to 14.1°C average monthly temperature, 150 to180 frost-free days, and an accumulation of 1600 to 1700 hours of cloud-free sunshine. During September, a 10 to15°C diurnal temperature change helps berries retain color, flavor, and acidity. The 110 to 200 mm seasonal rainfall is supplemented with irrigation water from the Yellow River. Alluvial soils vary in texture from sand to loam, with good drainage and moderate fertility. The land is generally flat or gently sloping. Since 1990, over 2,000 hectares have been planted with Cabernet Sauvignon, Cabernet franc, Merlot, Pinot noir, Shiraz, Chardonnay, and Riesling. These specific environmental conditions indicate that Ningxia is well-suited for winegrape growing.

Effects of Weed Management Practices on Aboveground and Seedbank Weed Communities in a California Vineyard

Lissa Veilleux* and Kendra Baumgartner

Sustainable Agriculture and Research and Education Program, University of California, Davis, One Shields Ave., Davis, CA 95616
[Fax: 530-758-5674; email: lmveilleux@ucdavis.edu]

California winegrape growers are under increasing pressure to decrease the use of preemergence herbicides in order to achieve water-quality standards established by various public regulatory agencies. Alternative weed control practices are available, but their effects on weed ecology are not known. In this study, eight alternatives to preemergence herbicides were evaluated for their effects on aboveground and seedbank weed communities. Research plots were established in a commercial vineyard in Oakville, CA, in a complete randomized block design with 5 blocks and 8 treatments. The following treatments were carried out on the berms: (1) fall cultivation + spring cultivation, (2) spring cultivation only, (3) fall cultivation + spring application of Round-up®, (4) fall cultivation + spring application of Matran™, (5) fall application of Round-up® + spring application of Round-up®, (6) fall application of Matran™ + spring application of Matran™, (7) cover crop of Idaho fescue (*Festuca idahoensis*), and (8) natural vegetation. Soil-quality characteristics and weed seedbanks were sampled in each treatment plot before treatments were applied, and weed biomass and species composition were assessed at spring peak biomass. Small differences in soil-quality characteristics and weed seedbank composition were found between blocks but not between treatment plots in the preliminary analysis. Differences in spring peak biomass were largest between the natural vegetation and the fall-cultivated and fall-applied herbicide treatments. There were smaller differences between the natural vegetation and cover crop treatments and between the cultivated and herbicide treatments.

* indicates corresponding author

Uptake of Legume Cover Crop Nitrogen by Winegrape Vines in the Central Valley of California

Jeanne M. Wiltberger, Rik Smith, Amy E. Patrick, Joyce E. Vivian, Kate Keck, and Alison M. Berry*

Department of Environmental Horticulture, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-1819; email: amberry@ucdavis.edu]

Leguminous cover crops such as vetch, clover, peas, and beans fix atmospheric nitrogen, adding net nitrogen to a vineyard nutrient budget through decomposition of mowed or tilled legume cover crop litter. This study traced nitrogen release from a legume cover crop mix and nitrogen uptake in winegrape vines, using stable isotope enrichment of the cover crop as a method. In no-till lysimeter studies, cover crop nitrogen was detected in grape leaves within two weeks of application, and a substantial fraction of grape leaf nitrogen was derived from the cover crop. In tilled field experiments, the time course of cover crop derived nitrogen release and uptake was similar, but the amounts taken up by the vines were substantially lower. Results from leaf and soil sampling, litterbag experiments, as well as whole vine studies, will be presented.

Evaluation of Grape Pollen Viability after Freezing in Liquid Nitrogen and Prolonged Storage at -80°C

L.E. Boyden* and P. Cousins

U.S. Department of Agriculture-Agricultural Research Service, Plant Genetic Resources Unit, Cornell University, N.Y. State Agricultural Experiment Station, Geneva, NY 14456 [Fax: 315-789-2339; email: leb17@cornell.edu]

In grape breeding, when crossing varieties whose flowering periods do not overlap, it is necessary to have a means of storing pollen for several weeks or months without loss of viability. It is also useful to have pollen available for making crosses in the greenhouse year-round. Pollen may be kept with a desiccant in a lab refrigerator or freezer, but loss of viability can be severe even for brief storage periods. Cryogenic pollen storage maintains adequate viability for longer periods of time, but requires specialized and expensive equipment. A new method was devised for storing grape pollen, by immersion in liquid nitrogen followed by storage at -80°C. Riparia Gloire, 1616C, and 3309C pollen was collected from dried flower clusters, cleaned of anthers and debris, and transferred to sterile microfuge tubes. The tubes were completely immersed in liquid nitrogen for 30 seconds, then immediately placed in a -80°C freezer. Three 300-grain pollen samples of each variety were tested for percent germination before flash-freezing, after flash-freezing and immediate thawing without storage, and after storage at -80°C for 2, 4, 6, 8, 10, and 12 months. Samples were incubated for 24 hr at 25°C in a 20% sucrose, 0.5% boric acid germination medium. Percent germination did not change significantly within varieties over the duration of the experiment. Results suggest that flash-freezing in liquid nitrogen and storage at -80°C is an effective method for maintaining pollen viability for at least one year and can be used to keep pollen readily available for breeding.

* indicates corresponding author

Response of Grafted Grapevines to Leaf Applications of 2,4-D

Peter Cousins*

Plant Genetic Resources Unit, U.S. Department of Agriculture-Agriculture Research Service, New York State Agricultural Experiment Station, Geneva, NY 14456
[Fax: 315-787-2339; email: psc9@cornell.edu]

Grapevines given exogenous auxin applications show symptoms similar to those of vines with fanleaf degeneration, including distorted leaves and reduced berry set. In fanleaf degeneration, symptom expression is related to the rootstock variety, with O39-16 reducing symptom severity. The symptoms, especially leaf distortion, that result from exogenous auxin application and from fanleaf degeneration may be related mechanistically. If so, then we may be able to use exogenous auxin applications to mimic the effect of fanleaf degeneration in a plant not infected with grapevine fanleaf virus, permitting further study of fanleaf degeneration and techniques for its management. To investigate the interaction between rootstock variety and exogenous auxin applications, 2,4-D solution was applied to the leaves of grapevines growing in a greenhouse. Green-growing benchgrafts of Sauvignon blanc were used. The rootstocks were SO4 and O39-16. A 10-µL droplet of 2,4-D solution in water was applied to one fully expanded leaf on each vine treated. Five vines on each rootstock were treated with each of four 2,4-D concentrations (10, 100, 500, and 1000 m/L). The 2,4-D solution included Tween 20 as a surfactant (0.1% v/v). Five vines on each rootstock received a 10-µL droplet of surfactant solution only and five vines on each rootstock were untreated. Leaf deformation was scored 36 days after 2,4-D application. Increasing concentrations of 2,4-D resulted in higher levels of leaf deformation, but there was no effect of rootstock variety on leaf deformation.

Forchlorfenuron and Ethephon Interact on the Berry Growth and Color Development of Flame Seedless Table Grapes

Nona C. Ebisuda and Nick K. Dokoozlian*

Department of Viticulture and Enology, University of California, Davis, and UC Kearney Agricultural Center, Parlier, CA 93648 [Fax: 559-646-6593; email: nkdokoozlian@uckac.edu]

Forchlorfenuron (N-(2-chloro-4-pyridyl)-N'-phenylurea) is a synthetic cytokinin that has significant physiological activity on many fruits, including grapes. When applied at fruit set forchlorfenuron stimulates both cell division and cell elongation, resulting in significant increases in berry size and a reduction in fruit color. Ethephon is plant growth regulator commonly applied to table grapes to enhance color development. A factorial experiment was conducted for two years (2000 and 2001) to examine interactions between forchlorfenuron (0, 3, 6, 9, and 12 g/ac) and ethephon (0, 0.75, and 1.5 pints Ethrel per acre; Ethrel = 22% ethephon) on the fruit growth and composition of Flame Seedless table grapes grown in the central San Joaquin Valley of California. Forchlorfenuron applied at fruit set increased berry weight approximately 15% compared to the untreated control, while berry diameter increased only slightly. Soluble solids declined with increased forchlorfenuron rate, while the number of harvestable clusters per vine dropped linearly as the rate of forchlorfenuron increased. Ethephon partially reversed the negative effects of forchlorfenuron on berry color development, with the greatest improvement observed at the highest rate. The percentage of clusters per vine that achieved adequate color for harvest ranged from 65% (0 forchlorfenuron + 1.5 pints Ethrel) to 7% (12 g/ac forchlorfenuron + 0 Ethrel). Berry firmness and capstem removal force increased linearly with forchlorfenuron concentration, while both parameters were negatively affected by ethephon.

* indicates corresponding author

Metabolic Profile of Berry Skin Flavonoids: Abiotic Stress Effects and Applications in Chemotaxonomy of Cultivated Grapes and Related Species

Alberto B. Iandolino,* Larry E. Williams, and Roger B. Boulton.

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA. 95616
[Fax: 530-752-0382; email: abiandolino@ucdavis.edu]

Flavonoids are a diverse familiy of C-based secondary metabolites with important biological functions. In grapes, different flavonoids accumulate during berry development and constitute the main determinants of organoleptic and health-promoting benefits of fresh grapes and wine. The variety and abundance of these secondary metabolites depend on genotype, viticultural practices, and environmental factors. Induction of flavonoid biosynthesis is responsive to abiotic stresses such as nutrient availability, vine water status, and UV light. The objectives of this study were to determine quantitative and qualitative changes in the flavonoid profile of Cabernet Sauvignon berries in response to multiple abiotic stresses, to characterize the diversity of flavonoids present in Vitaceae and cultivars of *Vitis* species, and to use skin flavonoid profiles as phenotypic markers for chemotaxonomic clasification and identification of cultivars. Trials conducted on field-grown Cabernet Sauvignon vines in a commercial vineyard near Oakville, CA, and on potted vines in a growth chamber confirmed that the main effects of abiotic stresses were on the total amount of flavonoids accumulated in the berry skin while the relative abundance of these compounds within a given family were maintained. Approximately 130 cultivars of *Vitis* and *Muscadinia* species from the vineyard blocks at the Foundation Plant Material Service, Davis, CA, and 30 different *Vitis* species and related genera from the Vitaceae family collected from the USDA-ARS National Clonal Germplasm Repository, Winters, CA, were sampled at fruit maturity in 2002. Skin extracts were analyzed by HPLC coupled with a diode array detector and mass spectrometer for chromatographic peak identification. The presence/absence and the relative abundance of different flavonoids were used as phenotypic markers for clustering and discrimination of the different cultivars and related species.

Genetic Dissection and Co-relation Analysis of Different Berry Skin Flavonoid Components of Cultivated European Winegrape (*Vitis vinifera* L.)

Alberto B. Iandolino,* Summaira Riaz, Larry E. Williams

Department of Viticulture and Enology, University of California, Davis
One Shields Ave., Davis, CA 95616
[Fax: 530-752-0382; email: abiandolino@ucdavis.edu]

We report the first study on the correlation and quantitative genetic analysis of grapevine berry skin flavonoids components. The quantitative trait locus (QTL) analysis was carried out on a consensus framework linkage map based on 154 microsatellite markers and one expressed sequence tag marker for *Vitis vinifera* L., the European winegrape. The mapping population consisted of 153 progeny plants from a cross of *Vitis vinifera* cvs. Riesling x Cabernet Sauvignon. This population has been selected as an international reference mapping population for grape genomic efforts. Flavonoids were extracted from the skin of mature grape berries and were analyzed by HPLC coupled with a diode array detector. The presence/absence and relative abundance of different flavonoids, mainly anthocyanins and flavonols, were used as phenotypic markers. QTLs for skin flavonoids would be identified with two different methods (interval mapping and Kruskal-Wallis rank sum test).

* indicates corresponding author

Juice and Skin Constituents Affected by Genetic Variation in *Vitis coignetiae* Pulliat Grapevines

Takashi Imai, Keiji Ueki, Denke Li, Goro Okamoto,* and Ken Hirano

Faculty of Agriculture, Okayama University
Tsushima-Naka, Okayama 700-8530, Japan
[Fax: +81-86-251-8388; email: gag14102@cc.okayama-u.ac.jp]

Vitis coignetiae grapevines, a wild-grape species originated in Japan, have been cultivated for red wine production in Hiruzen Heights, in western Japan. In a previous study, a wide range of amino acid contents in the juice suggested that genetic variations must exist among the vines. This study was aimed at elucidating the compositional characteristics in juice and skin of each vine in order to select the most useful vines for winemaking. Fifteen vines showing different morphological features were examined during the 2002 season. RAPD analyses with 50 primers allowed classification of the vines into four groups. Underside color of young leaves, greenish or reddish, could be used as a rough indicator to distinguish the vine groups. Skin anthocyanin composition, especially the level of malvidin anthocyanins relative to others, differed significantly among the vines belonging to different groups. The major amino acid in the juice was either proline, alanine, or glutamine, differing significantly depending on the group. Polyphenol content and free radical scavenging activity of the juice, assayed by the DPPH method, were widely different among the vines, although no consistent tendencies were found between the vine groups. Standards for selecting vines with the highest qualities were developed.

Leafhopper Management in California Vineyards

Tomé Martin-Duvall,* George Leavitt, Paul Verdegaal, Walt Bentley, and Lee Martin

University of California Cooperative Extension
328 Madera Avenue, Madera, CA 93637
[Email: tmmartinduvall@ucdavis.edu]

Grape leafhoppers and variegated leafhoppers are major pests in California vineyards, as they damage vines, vector diseases, and create problems for handcrews at harvest. Due to effective parasitism and chemical control of grape leafhopper species, populations have shifted primarily to variegated leafhoppers. Studies have shown that one variegated leafhopper can do 57% more tissue damage than one grape leafhopper. However, even with the potential losses related to elevated populations, monitoring is important and needs to be included in any management program. If economic thresholds are not reached, applications should not be made for leafhopper control, which may result in significant savings in this precarious market. Studies have been conducted over four years in infested vineyards utilizing a randomized complete block design. Leafhopper counts by species and nymphal stage were conducted prior to and 7, 14, and 21 days after application. In 2001, an additional study was conducted evaluating the efficacy of standards and new materials targeted for mite control on leafhopper control, with a number of materials showing excellent control of leafhoppers. Certain cultural methods such as leaf removal may also assist in reducing leafhopper populations below threshold levels.

* indicates corresponding author

Rooting Ability of Hardwood Cuttings of Norton Grapevine (*Vitis aestivalis*) under Different Conditions

Hiroyuki Matsui,* Masanobu Ushiyama, Katsuya Ohkawa, Hitoshi Ohara, Takahiko Soga, and Machiko Ochi

Faculty of Horticulture, Chiba University, Matsudo, Chiba 271-8510, Japan
[Fax: 81-47-308-8800; email: matsui@midori.h.chiba-u.ac.jp]

Propagation of *Vitis aestivalis* cultivar Norton by hardwood cuttings is difficult, with poor rooting being a major hindrance. The effect of indole-3-butyric acid (IBA) treatment and heating of propagation soil on the rooting of Norton cuttings compared to Cabernet Sauvignon and Merlot grapevines was examined. Canes were collected on 23 February 2002. Each treatment was administered to 30 single-bud cuttings, 10 to 15 cm in length. Cuttings were treated with IBA solution at 10, 50, or 100 mg/L and then planted (24 February) in wooden boxes filled with moist vermiculite. Soil temperature was maintained at 20°C and air temperature was 5 to 20°C. Another group was planted on 14 May and soil and air temperatures were not controlled. Results were obtained 77 and 48 days after cutting. Some canes were stored at -20°C for analysis of starch and phenolic compounds. In unheated soil, rooted cuttings of Norton and Merlot were 60 and 90%, respectively. However, the percentage of rooted cuttings treated with IBA was significantly higher than that of untreated control cuttings in Norton. Root number, length, and weight per cutting of both cultivars increased with increased IBA concentration. When the propagation bed was heated, it greatly promoted the rooting of Norton cuttings (97%), but there was no significant difference in the rooting of cuttings between IBA treatments. Higher contents of starch and phenolic compounds in the cuttings were observed in the order Merlot > Norton > Cabernet Sauvignon and Merlot > Cabernet Sauvignon > Norton, respectively. Results show that the propagation of hardwood cuttings in Norton is successful, with sprouting occurring after the promotion of rooting using a bottom-heated soil.

Comparative Analysis of the Economic Impact of Transgenic Grapevine Cultivars on the Wine Industry

Brady Smith*

Pennsylvania State University, Department of Economics
University Park, PA 16802
[Email: bps134@psu.edu]

Winegrape cultivars (*Vitis* sp.) are susceptible to an array of diseases and pests that threaten or prevent production in areas that are otherwise suited for cultivation. The phenotype of grapes used for wine production is highly dictated by cultural traditions and consumer demand. Use of traditional breeding methods to develop winegrape cultivars that exhibit resistance to a particular disease or pest is hampered by the heterozygous nature of grapevines and erosion of varietal traits. Introducing genes into winegrape varieties for resistance to diseases and pests, without diminishing distinct varietal characteristics, is difficult. Biotechnology methods of gene insertion hold promise of crafting cultivars that are highly resistant to diseases and pests but that maintain varietal fidelity. This method would satisfy the demand of consumers for the preservation of the unique traits of traditional winegrape varieties. During the past decade, corn (*Zea mays)* and soybean (*Glycine max*) industries have embraced the use of transgenic varieties. Introduction of transgenic varieties have led to an increase in the economic optimum yield and input switching technical changes. The counterfactual predicted risks and benefits of the use of transgenic corn and soybean varieties are compared with the observed effects. This model was used to examine the probability of occurrence of the perceived risks and benefits from transgenic winegrape production. A prediction of the impact on market structure and supply and demand for grapes and wines produced with transgenic cultivars is presented.

* indicates corresponding author

Automated Estimation of Grapevine Yields via Trellis Tension

Julie M. Tarara,* John C. Ferguson, Paul E. Blom, and Francis J. Pierce

U.S. Department of Agriculture-Agricultural Research Service, Horticultural Crops Research Laboratory, 24106 N. Bunn Rd., Prosser, WA 99350
[Fax: 509-786-9277; email: jtarara@wsu.edu]

Estimating yield in grapevines is labor intensive, producing both limited data and a static prediction. The standard method of crop estimation involves hand counting of clusters and berries early in the season, followed by later hand sampling of berries to determine average cluster or berry mass during the lag phase. A novel approach to estimate fruit mass uses automated measurements of tension in the main (cordon) wire of the trellis, which could increase labor and production efficiencies by providing real-time data from many sites. Its premise is that as the mass supported by the cordon wire increases, so does the tension in the wire. Thus, within-season changes in cordon wire tension can be used to make continuous estimates of growth and yield in grapevines and other trellised crops. A straightforward, annual calibration protocol has been developed. Results to date suggest a linear relationship between wire tension and fruit mass that varies among rows, but not within a row during a single season. Yields ranging from 2 to 9 tons per acre produced a similar response. Post-processing is used to account for the effects of wire temperature on tension, but with 5-second sampling and 15-minute data averaging, the effects of wind speed are minimized. Sensitivity to changes in crop mass declines exponentially with distance from the sensor. For well-watered vines with canopies that grow all season, vegetative mass must be separated from fruit mass. This requirement is less critical in deficit-irrigated winegrapes, with canopies that gain very little mass after fruit set.

Comparison and Optimization of RNA Extraction Methods for Grape Leaves

Elizabeth A.R. Tattersall,* Fadi AlKayal, John C. Cushman, and Grant R. Cramer

University of Nevada, Department of Biochemistry MS 200, Reno, NV 89557
[Fax: 775-784-1650; email: eat@unr.nevada.edu]

The high concentration of polyphenols and polysaccharides in grape leaves makes it challenging to obtain high-quality RNA from this organ. Many types 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, TRIzol™, and sodium perchlorate based methods. Some of these methods were tested on grape berries in addition to leaves. 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 quality RNA, suitable for PCR and other applications. In contrast, TRIzol™ and guanidine thiocyanate methods did not yield usable RNA.

* indicates corresponding author

Obtaining Usable Yields of High-Quality DNA from *Vitis vinifera* Suitable for Gene-Expression Studies

Douglas J. Walton* and Grant R. Cramer

University of Nevada, Reno, Department of Biochemistry MS 200, Reno, NV 89557
[Fax: 775-784-1650; email: dwalton@unr.nevada.edu]

In studying the response of *Vitis vinifera* to abiotic stress, the presence of secondary metabolites in the tissue such as polyphenols, polysaccharides, and proteins in mature tissue have made it difficult to obtain quality, high molecular weight DNA for molecular biology. Protocols exist that claim high yields of pure DNA from difficult plant tissues. We compared these different methods by subjecting the resulting DNA to enzymatic reactions as a further measure of purity. These assays included DNEasy™, cTAB (hexadecyltrimethlyammonium bromide) protocols, and chromatin IB. Both leaf and callus tissues were analyzed; some samples being lyophilized before use. Specific compounds were added to remove phenols and polysaccharides that interfere with downstream applications. Purity was assayed by spectrophotometric analysis, restriction enzyme digestion, in PCR reactions, and in Southern blotting. Samples varied in results based on protocol, age of tissue, and protocol used. Cost-benefit analysis was made for time, quality, total yield per gram of fresh tissue, and expense. Despite the time required for cTAB-based extractions, it has given the most consistent yield and purity.

Low-Temperature Exotherm Analysis: A New Bench-Top Technique

Robert L. Wample,* Denis Bacon, and Stephan Kidder

Department of Viticulture and Enology and Viticulture and Enology Research Center
California State University, Fresno, CA 93740
[Fax 559-278-4795, email: rwample@csufresno.edu]

A low-temperature exotherm system, designed and constructed to allow evaluation of cold hardiness of biological samples, had been developed. The computer-controlled system consists of a cooling chamber and 12 exotherm sensing modules. The insulated cylindrical chamber (37.5 cm diam x 3.5 cm deep) is cooled by passing a current through thermoelectric modules (TEM) attached to copper plates positioned on the top and bottom of the chamber. Heat removal is accomplished with a heat exchanger attached to the opposite side of the thermoelectric modules, which are connected to a refrigerated water bath. Temperature of the copper plates and the chamber is monitored by three thermisters. This computer-controlled cooling system attains low temperatures (0 to -30°C) and allows precise temperature ramping (4°C/ hr) with minimal hysteresis (<0.03°C). Each exotherm sensing module consists of a foam-lined plastic box (5 x 5 x 2.5 cm) containing a sample pressed against a TEM sensor. The TEM voltage (0 to 50 mV ±1.5 uV) generated by exothermic events is recorded with a 16-bit analog to digital A/D card and commercial software. Software permits real-time graphical monitoring of all sensor voltages and temperatures. Data can be transferred to other spreadsheets and graphics software for manipulation. Data from grapevine cane and bud exotherm analysis will be presented.

* indicates corresponding author

A New Proteolytic Enzyme for Wine Protein Removal

Daqing Sun,* Brian Lange, **Christina Beltrame**, and Jack Harris

Valley Research, Inc. 1145 Northside Blvd., South Bend, IN 46614
[Fax: 574-232-2468; email: dsun@valleyenzymes.com]

The current methodology for producing heat-stabilized white wine includes the use of bentonite to remove heat-unstable grape proteins from the wine. A proteolytic enzyme has been considered an ideal replacement for bentonite because it does not produce the problems that are incurred with bentonite usage, such as lowering wine quality, wine loss in lees and/or recovery costs, filtration problems, and waste disposal problems. A proteolytic enzyme has been developed and is now commercially available for this application. This enzyme has been shown to be effective in hydrolyzing heat-unstable proteins under standard winemaking and storage conditions. Additional benefits related to protein hydrolysis have been observed, such as reduced foaming in wine transfer, increased rate of fermentation at cold temperatures (possibly reducing the risk of stuck fermentations), and an increased rate of tartrate precipitation.

Selection of a New Malolactic Starter Culture for High-Alcohol Red Wines

Pia Bjerring* and Birgitte Kramp

Chr. Hansen A/S, Research, Development and Application, Wine Cultures,
Bøgeallé 10-12, 2970 Hørsholm, Denmark
[Tel: 45 45 74 7321; fax: 45 45 74 8816; email: pia.bjerring@dk.chr-hansen.com]

Malolactic fermentation (decarboxylation of malate to lactate) is a critical step in vinification and is in most cases performed by the malolactic bacteria *Oenococcus oeni*. Although many different strains of *O. oeni* are available, it is difficult and in several cases impossible to induce malolactic fermentation in some types of wine with extreme parameters (such as high alcohol, low pH). The production of high-alcohol red wines (alcohol >13 vol %) has increased in recent years. As no well-adapted starter culture is available for that segment, many winemakers have experienced problems with malolactic fermentation. Consequently, a project for the selection of *O. oeni* for high-alcohol red wine was initiated in 2000. Only a few of the isolated strains were able to survive inoculation and to grow and degrade the malic acid in the wines with elevated levels of alcohol. *O. oeni* strain CH16 showed remarkable performance in a laboratory trial performed with Pinot noir. At an elevated alcohol level of 14.7 vol % and pH 3.8, malolactic fermentation was completed within 20 days. Subsequently, the strain has been tested in red wine in both northern and southern hemispheres. In semi-industrial and full industrial-scale trials, the strain showed its superiority in high-alcohol red wines compared to other commercial available strains. The MLF was completed within 18 days in a Zinfandel with 15.1 vol % alcohol and pH 3.4 in a trial conducted at a northern California winery.

Sustainable Land Application Practices for Stillage and Nonstillage Process Water

Robert S. Chrobak*

Kennedy/Jenks Consultants, 622 Folsom, San Francisco, CA 94107
[Fax: 415-896-0999; email: bobchrobak@kennedyjenks.com]

A field investigation to determine best practices for sustainable land application of stillage and nonstillage winery process water was conducted on behalf of the Wine Institute. Field-test basins were prepared, including full monitoring instrumentation, at two wineries. Process water was applied at a range of flow rates and with varying lengths of wet/dry cycles. The soil and water loadings of nitrogen, biological oxygen demand (BOD), total dissolved solids (TDS), and other constituents were monitored over time. Based on field and analytical results, removal efficiencies were determined for various application and acreage scenarios, and these findings were used to formulate guidelines for sustainable management. The guidelines were presented to the Regional Water Quality Control Board to provide a scientific basis for potential modifications to their general waste discharge requirements.

Improving Wine Production and Compliance Practices by Using Software Technology

Kam Desai and Neil Kataria*

Riva Commerce Inc, 1001 Marshall Street, Suite 550, Redwood City, CA 94063
[Email: neil@rivacommerce.com]

Research on improving winery overall production process using software technology was conducted over a period of 18 months at all winery sizes, ranging from small, super-premium wineries to large, 10-million-case producing wineries. Each department within each winery was surveyed (winemaking, cellar, barrel, compliance, IT, scheduling), and then each part of the production supply chain, from vineyard activities to bottling line, was analyzed. Data was compiled, and the results were mapped onto a process diagram. Key findings included: improving software technology, making it a tool rather than a record-keeping system; moving the software technology to the center of the production process where all departments interact directly with the software across the wine enterprise; eliminating silos of information, whether they be with individuals or spreadsheets across the production supply chain; having one central software technology and one common data structure across the wine enterprise instead of in numerous disparate systems. With these and other findings, numerous process improvements in wine production can be realized.

New Instrumentation Solutions for Monitoring Soil Moisture and Improving Vineyard Management

Matt Galloway*

Decagon Devices, Inc., 950 NE Nelson Court, Pullman, WA 99163
[Tel: 509-332-2756 fax: 509-332-5158]

Recent availability of microcircuit technology has allowed the production of accurate and inexpensive sensors for use in vineyards and other horticultural industries. In vineyards, these sensors and instruments have proved to be useful for fine-tuning deficit irrigation and monitoring canopy conditions. As a result, both commercial and research vineyards are increasingly using Decagon ECH_2O probes and accessories for soil moisture monitoring and the AccuPAR PAR/LAI meter for quantifying light intensity and canopy density. With ECH_2O instrumentation, vineyard managers can gather and log soil moisture, temperature, and precipitation data over a larger sampling area to reflect the often diverse soil and climactic conditions that exist within a vineyard. AccuPAR can be used to both quantify the amount of photosynthetically active radiation (PAR) reaching different portions of the canopy and fruiting zone, and to calculate a target leaf area index (LAI) for monitoring vegetation growth. As a result of these new advances in technology and data collection, winery and vineyard owners now have more accurate and affordable options for implementing useful and productive vineyard management programs.

Comparison of Petiole Water Content and Petiole Water Potential in Syrah and Merlot

William Gensler* and Scott Thompson

Agricultural Electronics Corporation, P.O. Box 50291, Tucson, AZ 85703
[Fax 520-624-9720; email: wgensler@phytogram.com]

Vine water status can be assayed by an electrochemical measurement of petiole water content or by a physical measurement of leaf water potential. The relation between the two measurements in two blocks of Syrah and Merlot at Phillips-Hogue in Esparto, CA, were examined. Electrochemical sensors were permanently resident in petioles of 15 vines in each block. Measurements of water content by means of changes in sensor electrical capacitance were made every half hour from mid-June until harvest in late September. The criterion of water content was the daily minimum. Water potential measurements using a pressure chamber were made on five excised leaf petioles between 1200 and 1400 hours every other day from mid-June until harvest. Irrigation water was applied in accordance with anticipated ET requirements. Both water content and water potential decreased as the season progressed. For the combined blocks, water potential held at an almost constant value of -12.5 Mpa as the water content dropped from 50 and 35 Nfd/mm. Below a water content of 35 Nfd/mm, water content and water potential both decreased such that at 20 Nfd/mm the water potential was -1.55Mpa. The relation over a water content range from 50 down to 20 Nfd/mm can be described by the polynomial: $y = 0.0003\ x^3 - 0.036\ x^2 + 1.58\ x - 34.7$ where y is the water potential in Mpa *10 (bars) and x is water content in Nfd/mm.

The Virtual Vineyard: Canopy Simulation for Insolation Analysis and Trellis Design

David C. Luth* and Stuart B. Weiss

726 Eighteenth Ave. #4, San Francisco, CA 94121
[Email: david@eco-bizdecisions.com]

Selection of row direction and trellis design has a significant impact on solar exposure of grapes that directly influences wine quality. These research projects used hemispherical photographs of a vertical shoot position (VSP) trellis and various "free" shoots to simulate alternate trellis designs and row directions to address vineyard specific issues. These "virtual" trellises were then analyzed for direct sun exposure on the grapes to evaluate sunburn potential. For a Napa vineyard, the hourly insolation profiles for these virtual designs were overlaid with a typical heat wave hourly temperature profile to evaluate the effectiveness of the designs to reduce northwest exposure on the 25°NE row planting (first year with no trellis). Alternatives to the VSP trellis reduced the duration of peak insolation by up to 2 hours during the hot afternoon and reduced cumulative insolation over the growing season by 20% to 40%. A unique asymmetrical trellis design was chosen for this vineyard based on the results of these simulations. For a new vineyard planting in Oregon, alternatives to the traditional north-south row were evaluated and a 23°NE row was chosen with a VSP trellis. These virtual vineyard projects demonstrated the effectiveness of using Photoshop with hemispherical photographs to simulate the light environment of various trellis designs and row directions prior to vineyard planting and trellis selection.

Influence of Toasted Oak and Micro-oxygenation on Maturation of Cabernet Sauvignon

Jeffrey McCord*

StaVin Inc., P.O. Box 1693, Sausalito, CA 94901
[Fax: 415-331-0516; email: jeffmccord@stavin.com]

The Edna Valley grape-growing region located near San Luis Obispo, CA, is well known for the production of Pinot noir and Chardonnay. However, it is not suitable for the proper maturation of Cabernet Sauvignon. Orcutt Road Winery nonetheless was tasked each year to produce the best wine possible from these grapes for the bulk wine market. The question was asked if the use of toasted oak, micro-oxygenation, or the combination would allow the production of high-quality wines without the benefit of barrel aging. A trial was set in place to age wine in stainless steel tanks only, as control, and two types of toasted oak products, staves and segments, and each of these in combination with oxygen addition.

Application of Sensory Science Techniques to the Identification and Measurement of the Influence of Toasted Oak and Micro-oxygenation on Maturation of Cabernet Sauvignon

Leslie Norris*

FlavorSense, 139 Nantucket Cove, San Rafael, Ca 94901
[Fax: 415-453-1506; email: flavorsense@attbi.com]

A winemaking study to determine if the use of toasted oak, micro-oxygenation, or the combination would allow the production of high-quality Cabernet Sauvignon wines without the benefits of barrel aging was initiated by FlavorSense and Orcutt Road Winery. Difference panels and descriptive analysis panels were conducted to assist in the identification and measurement of the sensory changes that occurred with the different treatments. Sensory differences found among the treatments included vanilla, buttery, vegetative, leather, and tobacco aroma; fruity, buttery, and smoky flavor; and astringent aftertaste. In addition, the perception of color differed among the treatments. Correlations between the several of the sensory measurements and chemical measurements were examined.

Dried Starter Cultures of Non-*Saccharomyces* Yeasts for Alcoholic Fermentation: Impact of *Kluyveromyces thermotolerans* or *Torulaspora delbrueckii* with *Saccharomyces cerevisiae* on Aroma and Flavor Development in Wine

Peter Sommer,* Eva Stolpe, Birgitte Kramp, and Jens Heinemeyer

Chr. Hansen A/S, Research, Development and Application Wine Technology
10-12 Bøge Allé, DK-2970 Denmark
[Tel: 45 4574 7304; Fax: 45 4574 8816; email: peter.sommer@dk.chr-hansen.com]

Spontaneous alcoholic fermentation is the result of carbohydrate metabolism from a range of yeast species present in grape juice. Various secondary metabolic activities have the potential of achieving more complexity in wine. However, the delicate interspecies competition between the yeasts can lead to dominance of off-flavor producing species. This balance is determined by numerous factors, such as seasonal and geographical variations, grape variety, and basic hygiene conditions. Lack of consistency makes it difficult to rely solely on spontaneous alcoholic fermentation in modern winemaking. Introduction of starter cultures of *Saccharomyces cerevisiae* have given more process control in winemaking, but it is also claimed that it gives more uniform wines, lacking positive aroma and flavor contribution from a successful alcoholic fermentation by an indigenous yeast flora. The concept of mixing dried yeast starter cultures of *S. cerevisiae* and aroma and flavor-enhancing non-*Saccharomyces* yeast species to secure process control as well as aroma and flavor enhancement was tested in a series of experiments. Mixed dried yeast starter cultures of *Torulaspora delbrueckii* or *Kluyveromyces thermotolerans*, in combinations with *S. cerevisiae*, were tested in Pinot noir, Pinot blanc, and Chardonnay. Substrate use and product formation were monitored. Cell counts were conducted for both *S. cerevisiae* and non-*Saccharomyces* yeasts. Internal sensory evaluation of the wines produced was performed. NIF analysis and GC-MS were conducted for identification and quantification of aroma compounds. Non-*Saccharomyces* yeasts were restricted to the first part of the alcoholic fermentation, before they rapidly were outcompeted by the exponential growing *S. cerevisiae* yeast population. Sensory evaluations indicated that *T. delbrueckii* or *K. thermotolerans* in combination with *S. cerevisiae* gave a different aroma and flavor profile compared to alcoholic fermentation performed by pure strain *S. cerevisiae*.

* indicates corresponding author

Environmental Biophysics of Vineyards: Principles for Assessment and Design

Stuart B. Weiss* and David C. Luth

27 Bishop Lane, Menlo Park, CA 94025
[Email: stubweiss@netscape.net]

Vineyard canopies and grapes are subjected to variable fluxes of radiant, sensible, and latent heat that immediately determine local energy balances and ultimately determine the operative temperatures of both grapes and canopies over the course of growing seasons. Microclimate in a vineyard is driven by regional synoptic meteorology, modified by topographic features, and further modified by fine-scale structure of grapevine canopies. Geometric models of vineyard canopies developed with hemispherical photography define highly localized fluxes of direct light to grapes and the vineyard floor. These models can be embedded under an above-canopy atmospheric model driven by weather data and basic heat-transfer equations applied to estimate grape temperatures. Application of this analytic framework to vineyard design is demonstrated in a consideration of heat-damage potential under different trellis designs and row directions. Further applications include prediction of ripening rates and decoupling of canopy and cluster temperatures. Quantitative principles of environmental biophysics can be used for better matching macroclimate, topography, trellis, and varietals in planning and managing vineyards.