59
FIFTYNINTH
ANNUAL MEETING

SCIENCE

a platform for PROGRESS

59th
Annual
Meeting

June 17–20, 2008

Oregon Convention Center
Portland, Oregon, USA

TECHNICAL ABSTRACTS

AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

MARK YOUR CALENDAR:

NAPA VALLEY

june 22–26, 2009

ASEV will celebrate its 60th Annual Meeting by debuting a new venue. Research reports, supplier-industry seminars and themed symposia—all capitalizing on wineries, vineyards and suppliers of Napa Valley.

Immerse yourself in our new content-driven program while networking with experts from vineyards and wineries of this famed wine region—an ideal opportunity for every segment of the wine industry.

SCIENCE

a platform for PROGRESS

60th Annual Meeting

June 22–26, 2009

Napa, California USA

SPECIAL EVENTS

High Ethanol Wine Symposium

Rootstock Symposium

Winery Tours

www.asev.org
530-753-3142

American Society for Enology and Viticulture

2008 Technical Abstracts Review Committee

Michael Silacci (Chair), Opus One

Linda Bisson, University of California, Davis

Jean-Emmanuel Danjoy, Opus One

James Harbertson, Washington State University

Nathalie Jure, Opus One

John Thorngate, Constellation Wines US

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

Alphabetical Listing of Presenting Authors

Agosin, E.	38	Logan, G.	40, 53
Anderson, M.	19	Mannino, M.	28
Battany, M.	19	Martínez-Diaz, G.	24, 24
Benkwitz, F.	33	Mendes-Ferreira, A.	12
Bowen, A.	45	Meyers, J.	25
Cahill, K.	5	Mueller, J.	25
Cohen, S.	41	Musingo, M.	13
Conversano, M.	1	Oskwarek, L.	13
Cousins, P.	20	Peterson-Nedry, W.	55
Danilewicz, J.	43	Petrovic, S.	14
Downey, M.	1, 2, 40	Poudel, P.R.	26
Drayton, W.	51	Qian, M.	33
Du, X.	5, 6	Robinson, S.	37
Durner, D.	43	Rodrigues, D.	50
Elias, R.	6	Rowe, J.	37
Fischer, U.	7, 46	Rust, A.	14
Forester, S.	55	Ryona, I.	35
Fredrikson, L.	20	Saito, S.	31
Gu, S.	21, 35	Sampaio, T.	4, 53
Hang, Y.	7	Scholasch, T.	29
Hanlin, R.	41	Skogerson, K.	15
Harbertson, J.	2, 54	Smith, R.	26
Heldt, A.	3	Sommer, S.	15
Hernández-Jiménez, A.	21	Sorokowsky, D.	48
Hinckley, E.	48	Spayd, S.	51, 52
Hirson, G.	46	Strever, A.	49
Hirzel, D.	8	Suzuki, S.	27
Holler, M.	22	Tarara, J.	42
Hong, Y.	9	Trela, B.	16
Howe, P.	9	Ugliano, M.	45
Inês, A.	10	Vasconcelos, C.	36
Jordan, B.	23	Walker, A.	30
Joseph, C.M.L.	10, 47	Walsh, J.	16
Kennedy, J.	54	Weaver, R.	17
Kilmartin, P.	11, 34	Weissberg, L.	38
Kim, J.I.	11, 12	Wilcox, W.	30
Krasnow, M.	31	Wilker, K.	17
Krawitzky, M.	23	Williams, L.	49
LaBarge, P.	4	Willwerth, J.	52
Lebrun, S.	28	Zhang, X.	18

Abstracts here are those submitted and accepted through the 2008 Call for Papers only.

Sessions & Presentations Listing

Wednesday, June 18 and Thursday, June 19

Enology/Viticulture Poster Session	1–4
Enology Poster Session	5–18
Viticulture Poster Session	19–27
Industry Poster Session	28–29

Wednesday, June 18

Plant Disease Session	30–32
Aroma Chemistry	33–34
Vine Management Session	35–36
Wine Microbiology Session	37–39
Berry Development Session	40–42
Oxidation Session	43–44

Thursday, June 19

Sensory Session	45–47
Water Relations Session: Part I	48–50
Water Relations Session: Part II	51–52
Phenolics Session	53–56

Abstracts are in the approximate order as noted in the conference program. Abstracts here are those submitted and accepted through the 2008 Call for Papers.

Role of Fruit Maturity on Winemaker Harvest Decisions for Cabernet Sauvignon Wines

Michael Conversano,* Marco LiCalzi, Kirsten Skogerson, Mark A. Matthews, Hildegarde Heymann, and Susan E. Ebeler
Department of Viticulture and Enology, University of California, Davis, CA 95616 [mrconversano@ucdavis.edu]

How does fruit maturity influence the harvest decisions of winemakers and their perceptions of the resulting wines? Sensory and winemaker "quality" analyses were conducted on fruit harvested at different maturities and on wines made from these fruit. In 2006 and 2007, winemakers from the Napa Valley were asked to determine whether grapes harvested from Paso Robles, which were picked between 21 and 30.8 Brix, were ready to be harvested and to rate the quality of the fruit. For both years, grapes at 21 to 24.7 Brix were unanimously deemed not ready for harvest, and grapes from 26 to 30.8 Brix were rated increasingly ready to harvest, indicating that the longer the grapes hung on the vines, the more certain the winemakers were that the grapes were ready to be picked for winemaking. In contrast, the grape quality score peaked with the fourth harvest for both years, coinciding with 26 Brix. In July 2007, winemakers evaluated wines made from the 2006 fruit. Results indicated that the winemakers thought the wines made with grapes harvested between 24.7 and 26 Brix were of better quality, were picked at the correct ripeness, and should be priced higher than wines made with earlier or later harvested fruit. Thus this study indicates that the winemakers judged the fruit as most ready for harvest when it was above 30 Brix, but awarded the highest quality scores to the fruit and the resulting wines harvested between 24 and 26 Brix.

Comparison of Methods for Analyzing Tannin in Grape Skin

Tara J. Seddon and **Mark O. Downey***
Department of Primary Industries Victoria, Mildura, VIC 3502, Australia [mark.downey@dpi.vic.gov.au]

Tannins are polymers present in grapes that contribute to the color and mouthfeel of red wine. Tools to monitor tannins during berry development and winemaking are necessary for improving wine quality. While numerous methods exist for tannin analysis, many are limited by lack of specificity, long processing times, or require costly equipment. Methods attracting increasing interest from the wine industry include protein precipitation, methylcellulose precipitation, and acid catalyzed depolymerization and quantification by high-performance liquid chromatography (phloroglucinolysis). In selecting a method for routine tannin analysis, we compared the level of tannin determined by each of these methods in the skin of 36 different grape cultivars. Grapes were collected at commercial harvest from vineyards in Sunraysia (VIC, Australia) and included both wine and table grapes. Total phenolics were also measured and compared with the level of tannin. No correlation was found between phenolic and tannin levels, suggesting the ratio of tannin to nontannin phenolics is highly variable between cultivars. Neither was there a correlation between tannin levels determined by phloroglucinolysis and by protein or methylcellulose precipitation. It is apparent that each method measures a different component of

the total tannin in grape skin. The disparity between methods means that method selection should reflect subsequent decision-making, for example, precipitation of tannins by protein has been strongly correlated with perceived astringency; consequently, if an indicator of potential mouthfeel is important to management decisions, that may be the most appropriate method.

Tannin Biosynthesis in Chardonnay and Cabernet Sauvignon Grape Skin

Allison S. Hogg, Jochen Bogs, Anthony R. Gendall, Simon P. Robinson, and **Mark O. Downey***
Department of Primary Industries Victoria, Mildura, VIC 3502, Australia [mark.downey@dpi.vic.gov.au]

Condensed tannin accumulation and the expression of genes encoding the enzymes required for tannin synthesis have only been studied extensively in Shiraz grape berries. We examined the hypothesis that the pattern of tannin accumulation and tannin biosynthesis reported in Shiraz was typical of other grape cultivars. The content and composition of condensed tannins in the skins of Cabernet Sauvignon and Chardonnay grape berries over the 2002–2003 developmental season was analyzed by HPLC following acid-catalyzed depolymerization in the presence of excess phloroglucinol. Expression of genes encoding enzymes involved in condensed tannin biosynthesis, leucoanthocyanidin reductase (*VvLAR1* and *VvLAR2*), leucoanthocyanidin dioxygenase (*VvLDOX*), and anthocyanidin reductase (*VvANR*) was analyzed by real-time PCR. Condensed tannin accumulation in the skins of Cabernet Sauvignon and Chardonnay berries was highest at fruit set in both varieties and gradually decreased toward harvest. The major extension subunit of both cultivars was epicatechin, while terminal subunits were primarily catechin. Expression patterns of *VvLAR1* and *VvLAR2*, *VvLDOX* and *VvANR* were similar in both varieties. While subunit composition in Cabernet Sauvignon and Chardonnay grape skins was similar to that reported in Shiraz, the timing of tannin accumulation was significantly different, with the peak in tannin accumulation occurring around the time of fruit set rather than at veraison as previously reported. Transcript accumulation of *VvLAR1* and *VvLAR2*, *VvLDOX* and *VvANR* correlated well with the pattern of subunit accumulation. These observations suggest that while the timing of tannin accumulation in grapevines is variable, probably in response to environment, transcriptional regulation of tannin biosynthesis is universal in grapevines.

Survey of Merlot and Cabernet Sauvignon Grape Constituents from Washington State

James F. Harbertson,* Maria Mireles, and Sara Spayd
Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350 [jfharbertson@wsu.edu]

During the 2002–2003 seasons, Merlot (MR) and Cabernet Sauvignon (CS) were harvested in sync with commercial harvest from 26 blocks located at eight vineyard sites in the Columbia Valley. Yield differed between years by about 3 kg/vine and did not differ between cultivars. Yield was similar between east and west sides, but tended to be slightly lower in 2002 on the west side due to a tendency for fewer

clusters. MR (2003) berries were larger than CS and 2002 berries, respectively. Seed weight/berry was higher in 2002 than 2003, despite no difference in seed number/berry. CS and MR averaged 1.8 and 2.0 seeds/berry, respectively. Average seed weight/berry for CS was 61 mg/berry and MR was 76 mg/berry. Soluble solids concentration at harvest for both years and cultivars averaged between 25.1 and 25.5%. Fruit pH was higher in 2003 than 2002 and for fruit from the west side than the east side of the canopy. Across vintages and cultivars and all locations, aspect did not affect the concentrations of either tannin or anthocyanins, although there was a trend for fruit from the east side to have higher levels of all fractions. Merlot berries had a greater concentration of anthocyanins while Cabernet Sauvignon had greater skin tannin. When vineyards were compared we found differences in anthocyanins, skin tannins, but not in seed tannins. Differences were also seen between vintages. Although other viticultural variables were collected, we saw no immediate relationship to explain the phenomenon.

Characterization of the Antimicrobial Activity of Phenolic Extracts in California Grape Pomace

Amanda Heldt and Linda Bisson*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [lfbisson@ucdavis.edu]

Grape pomace, which is composed of the grape's skins, seeds, and stems is generally viewed as a waste byproduct of wine production and is usually disposed of immediately. What has been underestimated, however, is the amount of phenolic compounds that remain bound in pomace when discarded. Due to the increasing interest in finding natural alternatives for the preservation of food, preliminary studies investigating the potential use of fruit and wine phenolics as potential antimicrobials to reduce the incidence of food spoilage caused by bacterial contamination has been widely explored across Europe and Asia. The purpose of the present study is to replicate the observed antimicrobial activity found in phenolic-based pomace extracts from cultivars grown in California, and to explore the scope of inhibition as it relates across microbes and individual phenolic compounds. Pomace was collected, dried, and phenolics were extracted with acetone/water (70:30). Total phenolic content was determined by the Folin-Ciocalteu method, and Montepulciano and Grenache varieties were 83.95 mg GAE g^{-1} and 17.95 mg GAE g^{-1}, respectively. Inhibitory effects of phenolic extracts were assessed in LB nutrient broth against *E. coli* DH10B at 0, 0.5, 1.25, 2.5, 5, and 10% (w/v) concentrations. Bacteria tested were inhibited at 5% and higher concentrations after 48 hr, while extracts seemed to exhibit bacteriostatic effects at concentrations of 2.5% after 24 hr.

Influence of Canopy Management on Cabernet Sauvignon Grapes and Wine

Pierre LaBarge IV and Keith Patterson*
Department of Horticulture and Crop Science, California Polytechnic State University, San Luis Obispo, CA 93401 [kpatters@calpoly.edu]

Maximizing quality in Cabernet Sauvignon requires clarification of the role and possible interaction between crop level and light in the fruit zone. Cabernet Sauvignon vines in Paso Robles, CA received shoot-thinning treatments at bloom, leaf-pulling treatments at fruit set, and cluster-thinning treatments at veraison. Berries were analyzed chemically and the wines will be subjected to a sensory panel based on preference. Vine treatments subjected to leaf removal had higher total phenolics, anthocyanins, and color density, regardless of the crop load. Reduction of crop load without leaf removal had little effect on the total phenolics, anthocyanins, and color density. 3-Isobutyl-2-methoxypyrazine content was less than 5 ng/kg for all treatments and not affected by vine manipulation. Also, Brix, TA, pH, malic, and tartaric acid were not significantly affected by either crop reduction or leaf removal. Reduction of crop load may not be necessary for improving quality of Cabernet Sauvignon wine as long as there is adequate leaf removal and light in the fruit zone.

Effect of Rootstock on Fruit and Wine Composition

Tiago Sampaio, James A. Kennedy, and M. Carmo Vasconcelos*
HortResearch Marlborough, Blenheim 7240, New Zealand [carmo@hortresearch.co.nz]

Rootstocks have been used to overcome phylloxera, nematodes, site environmental constraints, and to control plant size. Little is known about their effect on vine physiology, and there are no reports on their impact on fruit and wine phenolic composition. The purpose of this study was to provide a better understanding of the role that rootstocks have in determining fruit and wine chemistry. A rootstock trial was established at Oregon State University's research vineyard in 1997. Pinot noir was grafted onto 19 different rootstocks and compared to ungrafted vines in a completely randomized block design. Sugar levels across all rootstocks were very similar, provided that rootstocks achieved an adequate leaf area to fruit ratio, suggesting that rootstock growing cycles are not necessarily different. Juice titratable acidity increased with vegetative growth. Skin tannin concentration was independent of the vigor imposed by the different rootstocks and was highest for 125AA, 1616C, and 420A and lowest for 161-49C, Schwarzmann, 3309C, and Riparia Gloire (mg/berry). Rootstocks SO4, 8B, 420A, and 140Ru had higher anthocyanin concentrations. Differences in berry weight were not related to the concentration of skin tannin or total anthocyanin (mg/kg fruit) found. The wine phenolic profile did not entirely reflect fruit composition, since the extraction of skin components into wine varied greatly with rootstock. These results suggest that there was a direct rootstock effect on fruit and wine chemistry, rather than a consequence of differences in crop size, vine vigor, or berry weight.

Recommended Grape Preparation Methods for Fast, Accurate Results with the Harbertson–Adams Phenolic Assay

Kimberly Nicholas Cahill* and Mark A. Matthews
Department of Global Ecology, Stanford University, Stanford, CA 94305 [kncahill@stanford.edu]

The Harbertson–Adams assay has become widely adopted to achieve rapid and relatively low-cost measures of phenolic compounds important in wines. The method has also been applied to grape extracts using diverse preparation methods, including varying approaches for separating grape skins from the rest of the berry, preparing skins for extraction, and extraction technique. These sample preparations were compared and the resultant differences in the measured levels of anthocyanins, tannins, and total iron-reactive phenolics are reported. We conclude that significantly higher (and presumably more representative) readings are obtained from peeling rather than crushing fruit, using fresh skins rather than freeze-dried ground skins, and exposing skins to liquid nitrogen before extraction rather than a control without liquid nitrogen. Samples extracted in 50% ethanol had significantly higher anthocyanins but lower tannins than those extracted in 70% acetone. However, the correlation between the two extraction methods was good (>0.80), and samples extracted in ethanol had good results without the need to evaporate off the extract, which offered significant time savings. We also report on the correlations between paired methods to offer a potential for comparison between studies and a metric for evaluation of time vs. accuracy trade-offs in grape preparation methods.

Analysis of Aroma and Aroma Precursors in Pinot noir Grapes Using Stir Bar Sorptive Extraction–GC-MS

Xiaofen Du and Michael Qian*
Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331 [michael.qian@oregonstate.edu]

The aroma of Pinot noir wine is often considered one of the most complex of all varietal wines. Generally, the aroma potential of a wine derives from free aromatic volatiles in grapes and nonvolatile precursors which could be hydrolyzed during winemaking, and analysis of these compounds is of particular interest to winemakers. Stir bar sorptive extraction (SBSE) is a recently developed sample preparation technique, and it can efficiently extract trace and ultratrace quantities of aroma compounds in food samples. The technique was used to analyze aroma and aroma precursors in Pinot noir grape, on which there is little research. Free aroma compounds were extracted with stir bar by stirring in must containing 30% sodium chloride for 3 hr and then thermally desorbed onto a GC-MS for analysis. Aroma precursors were isolated with C18 SPE column, hydrolyzed by enzyme for 24 hr in 0.2 M citric acid buffer at pH 3.1, and then freed aglycones were analyzed with SBSE-GC-MS. Approximately 50 aroma compounds were identified, including 17 norisoprenoids. α-Isophorone, 4-ketoisophorone, β-cyclocitral, *trans*-β-damascenone, and geranyl acetone were identified in both free and precursor forms. Safranal, α-ionone, 5,6-epoxy-β-ionone, β-ionone, dihydroactinidiolide, and 4-oxo-β-damascenone were identified only in free form, while high concentrations of vitispirane, TDN, *trans*-β-damascenone, hydroxydihydroedulan, megastigmatrienones, and 3-hydroxy-β-damascenone existed

in the form of precursors. The method is sensitive, reproducible, and could be used to evaluate the potential quality of grapes.

Comparison of Mild Enzymatic Hydrolysis and Acidic Hydrolysis on Monoterpenoic and Norisoprenoidic Glycoconjugates in Pinot noir Grapes

Xiaofen Du, Changrong Ou, and Michael Qian*
Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331
[michael.qian@oregonstate.edu]

The specific varietal aroma profile of a wine arises from specific combinations of odor-active compounds. Monoterpene alcohols and C_{13} norisoprenoids play very important roles in varietal aroma. However, the majority of these compounds are present in the grapes as bound glycosidic precursors. Although these precursors themselves are odorless, they could be hydrolyzed by enzyme or acid during wine-making or aging. However, reliable analysis of these bound precursors is extremely difficult because many of these compounds are unstable and can be easily transformed to other compounds during hydrolysis. The objective of this study was to compare mild enzymatic hydrolysis and acidic hydrolysis of aroma precursors in Pinot noir grapes. The flavor precursors were isolated from Pinot noir grape must with C18 SPE column, and then hydrolyzed either by Macer8 FJ enzyme in 0.2 M citric acid buffer at pH 3.1 at 45°C, or in 0.2 M citric acid buffer at pH 2.5 at 100°C. The freed aglycones were analyzed with SBSE-GC-MS. The results demonstrated that the aglycone composition was dependent on hydrolysis condition. Enzymatic hydrolysis generated many monoterpene alcohols such as *cis*-linalol oxide, *trans*-linalol oxide, linalool, citronellol, nerol, and geraniol, while mild acidic hydrolysis gave mostly neroloxide. The levels of the C_{13} norisoprenoids such as vitispirane, 1,1,6-trimethyl-1, 2-dihydronaphthalene, β-damascenone, hydroxydihydroedulan, 3-hydroxy-β-damascenone, and megastigmatrienones were proportional to hydrolysis time during the enzymatic hydrolysis. Acidic hydrolysis resulted in much greater amount of these C_{13} norisoprenoid compounds.

A Method for the Accurate Measurement of Free and Sulfite-Bound Wine Carbonyls by HPLC

Ryan J. Elias, V. Felipe Laurie, Jon W. Wong, Susan E. Ebeler,
and Andrew L. Waterhouse*
Department of Viticulture and Enology, University of California, Davis, CA 95616
[alwaterhouse@ucdavis.edu]

The compounds acetaldehyde, formaldehyde, 2-ketoglutaric acid, glyceraldehyde, and pyruvic acid are of great importance to wine quality, particularly with respect to color and aroma, and are potentially useful oxidation markers. Here we describe an HPLC method for the quantitation of the above carbonyls and their sulfite-bound (α-hydroxysulfonate) forms in wine. At present, most published procedures to release sulfite-bound carbonyls in wine use alkaline conditions to dissociate the α-hydroxysulfonate forms. In this study, we demonstrate for the first time that this alkaline hydrolysis step increases the concentration of carbonyls in wine samples,

most likely due to the accelerated oxidation of phenolic compounds. This base-catalyzed oxidation leads to an artificially high reporting of endogenous carbonyls, such as acetaldehyde. Here we propose a solution based on a simple oxygen exclusion step using nitrogen gas. Sulfite-bound carbonyl compounds, in both model solutions and wines, were dissociated at alkaline pH under anoxic conditions, rapidly derivatized with 2,4-dinitrophenylhydrazine, separated on a C18 column by HPLC, and analyzed with a diode array detector. The technique offers good specificity, reproducibility, accuracy, and limits of detection. The method was successfully used to measure concentrations of carbonyls in both red and white wines, as well as model wine solution containing a large excess of sulfur dioxide.

Implementation of FT-MIR in Wineries to Measure Grape Composition

Ulrich Fischer* and Thomas Berger
DLR-Rheinpfalz, Department of Oenology and Viticulture, D-67435 Neustadt/Weinstrasse, Germany [ulrich.fischer@dlr.rlp.de]

Commerce in grapes and grape must leads to the challenge of objective pricing for this product. FT-MIR spectrometry offers the possibility of rapid evaluation of quality and ripeness of the delivered grapes during harvest. In less than 2 min, more than 10 different parameters can be analyzed. Analyzing a high number of grape samples over three vintages by reference methods and FT-MIR, a robust calibration was obtained even for minor compounds such as α-amino nitrogen or volatile acidity. Analyzing grapes that were completely infected by *Botrytis cinerea* but that varied in length of infection showed substantial deviation regarding chemical composition. Therefore the sanitary aspects were not characterized by a percentage of infected grapes, but by a chemical definition of the metabolic impact of the mold, including ethanol, glycerol, gluconic acid, volatile acidity, and glucose-fructose ratio. For nitrogen supply in grape juice, FT-MIR analysis yields information about ammonium as well α-amino nitrogen content. Furthermore, a calibration was developed that also allows monitoring the consumption of nitrogen by microorganisms during fermentation parallel to sugar degradation and potential rise of lactic and volatile acidity. The implementation of FT-MIR in the grape reception area allows wineries purchasing grapes a much closer and more detailed look at grape composition. Data from FT-MIR analysis can be incorporated in quality control, traceability schemes, and even payment systems to grapegrowers. Stronger consideration of grape quality will give growers more incentives to produce sound and superior grapes. Thus FT-MIR analysis may contribute significantly to a more objective and fair trade of grapes.

Limiting the Formation of Methanol in Wines and Distilled Fruit Spirits

Yong D. Hang* and Edward E. Woodams
Department of Food Science and Technology, Cornell University, Geneva, NY 14456 [YDH1@cornell.edu]

Methanol in wines and distilled fruit spirits is a product that results during fermentation from the hydrolysis of fruit pectin by pectic enzymes. The U.S. Bureau of Alcohol, Tobacco, Firearms and Explosives has set a tolerance methanol level of

0.35% by volume for brandy and other distilled fruit spirits. It is thus important to use only the process that can produce distilled spirits with the concentration of methanol within the legal limit. This research was undertaken to determine if the heat inactivation of pectic enzymes would minimize the formation of methanol in wines and distilled fruit spirits. The methanol concentration of distilled spirits produced in the current study from nonpasteurized apple juice, for example, was greater than 0.45% by volume, but the distilled spirits from pasteurized apple juice contained only 0.26% methanol by volume. Similarly, we also discovered that the distilled spirits produced from pasteurized pomace had a significantly lower level of methanol than similar products derived from nonpasteurized pomace. The results of the present investigation clearly indicate that the heat inactivation of pectic enzymes in fruit juice and pomace prior to alcoholic fermentation could significantly limit the formation of methanol in wines and distilled fruit spirits.

Development of a Fluidized Bed for Crystallizing Potassium Bitartrate from Wine

David R. Hirzel* and Roger B. Boulton
Department of Viticulture and Enology, University of California, Davis, CA 95616 [drhirzel@ucdavis.edu]

A bench-scale, fluidized bed was developed to crystallize potassium bitartrate (KHTa) from wine. Bitartrate crystals that precipitate from wine after bottling are considered a flaw by most consumers. Traditionally wine is cold stabilized by maintaining the wine at just below freezing temperatures for several weeks to promote bitartrate precipitation. In the fluidized bed, chilled wine was passed through a bed of KHTa seed crystals to promote rapid crystallization. The fluidized bed was potentially a better alternative, since only the wine in the bed is chilled, it requires no additional process water, and recovers the bitartrate without filtering. A plexiglas column (i.d. 21 mm, L = 900 mm) was constructed. The system was insulated to minimize energy losses during operation. Conductivity probes and thermocouples collected data during the trial runs. Tartrate removal was tracked by measuring the change in conductivity as potassium ions precipitated out of a young white wine. A heat exchanger reclaimed some of the energy used to cool the wine by contacting the cold product with the fresh feed. Treated wine was returned to the feed tank and layered across the bottom of the tank without significant mixing with the untreated feed. Runs lasting up to 93 hr were performed with little noticeable settling of the bed or degradation of performance. The fluidized bed was able to reduce the conductivity of the wine by 640 µS/cm and traditional cold stabilization only reduced the conductivity by 270 µS/cm. The heat recovery system reduced energy consumption by >40%.

Impact of Modification of the MET10 Allele on Fermentation Performance in *Saccharomyces*

Yeun Hong, Angela Linderholm, and Linda F. Bisson*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [lfbisson@ucdavis.edu]

Hydrogen sulfide (H_2S) produced by yeast during alcoholic fermentation confers an undesired off-odor of rotten eggs to wine and has been implicated in causing the appearance of other sulfur-containing off-odors during aging or in bottle. Derivatives of the high H_2S producing strains UCD522, UCD940, and UCD950, carrying a single gene modification at the *MET10* locus, were created. Hydrogen sulfide production in these strains was eliminated by this single genetic change. The aim of this study was to determine if this genetic alteration led to any other change in the enological or biological traits of these derivatives as compared to their parental (unmodified) strains. Sensitivity to sulfite was examined as variation of the *MET10* allele may lead to a modification of sulfite reductase activity in vivo. All three derivatives showed same growth as their parental strains under the different SO_2 concentrations. Growth and fermentation ability were compared for the modified and unmodified strain pairs singly and by using a mixed culture of modified and unmodified UCD522. The unmodified strain occasionally showed a slight growth advantage in mixed culture over the modified strain but there was no statistically significant difference.

Quantification of Effects of Common Variables on Determination of Volatile Acidity in Wine

Patricia Howe,* Susan E. Ebeler, and David E. Block
Patricia Howe Wines, Napa, CA 94581 [pathowe@pathowe.et]

Use of the modified Cash steam still for the determination of volatile acidity in wines is an AOAC standard method and is used by the TTB as a reference method. Many published wine analysis textbooks have adapted the AOAC procedure, so few wineries follow the validated procedure. In addition, many modifications have been introduced both in equipment and method. Most recently, the control of SO_2 through back-titration versus several methods of oxidation was evaluated. However, earlier AOAC validation studies found lactic acid carryover, the pH of the sample, sample size, distillate volume and other factors could significantly affect results, but researchers did not quantify these effects. A single laboratory validation was performed of the reference method, testing not only variables existing within the sample (such as acetic, lactic, succunic, sorbic, carbonic and sulfurous acid, in addition to the actual pH of the sample) but also variables in performing the test (sample volume, distillate volume, condenser/distillate temperature, and boiling rate). Prepared samples testing extremes of the variables were sent to several commercial winery laboratories to establish which variables are the most common sources of error in the industry.

Screening Enzymes with Enological Relevance in Lactic Acid Bacteria Isolated from Douro Wines

António Inês, Tânia Tenreiro, Rogério Tenreiro, and Arlete Medes-Faia*
IBB-Centro de Genética e Biotecnologia, Universidade de Trás-os-Montes e Alto Douro, 5001-813 Vila Real, Portugal [afaia@utad.pt]

A representative sample of a lactic acid bacteria collection isolated from Douro wines that were spontaneously undergoing malolactic fermentation was studied using phenotypic and molecular characterization. As bacterial strains associated to wine have to be beneficial without any detrimental effect on wine quality and safety, our main goal was to survey some specific enzymes considered valuable to obtain a high-quality product. Thus, arginine deiminase pathway, ß-glucosidase, histidine, and tyrosine decarboxylases among others were screened in all isolates. The enzymatic activities were assayed in differential culture media and were confirmed by gene specific PCR. The identity of the amplified products was confirmed by sequencing for *bgl*, *mle*A, *arc*AC, and *tyr*DC genes. Desirable characteristics like the presence of ß-glucosidase and absence of arginine deiminase, histidine, and tyrosine decarboxylases were detected in 30% (69 of 227) of oenococci, in 36% (15 of 42) of lactobacilli, and in 25% (6 of 24) of pediococci. The results reinforce the use of these tools for selection of starters. Preliminary expression studies (RT-PCR assays) for *arc*AC and *tyr*DC genes, in order to evaluate how these genes could be regulated by some abiotic factors (pH, ethanol and malic acid), were performed in one strain of *Oenococcus oeni* and one of *Lactobacillus brevis*.

Diacetyl Production by *Lactobacillus casei* Isolated from Wine

Angela Marcobal, **C.M. Lucy Joseph**, Valentina Canuti, Susan E. Ebeler, and David A. Mills*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [damills@ucdavis.edu]

Diacetyl is a buttery flavor compound produced mostly by lactic acid bacteria in wine. We identified a number of spoilage-associated *Lactobacillus casei* strains that produced a significant buttery aroma indicative of diacetyl. The level of diacetyl produced by these strains, grown semi-aerobically and anaerobically, was determined by solid phase microextraction gas chromatography-mass spectrometry. Interestingly, seven of nine *L. casei* strains produced higher concentrations of diacetyl than that observed from *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, a strain specifically used in dairy fermentations for diacetyl production. Moreover, two of the wine isolates produced significantly more diacetyl than that observed from a *L. casei* strain originally isolated from cheese. The strains were further examined via carbohydrate assimilation tests and multilocus sequence typing. These methods revealed a relationship among diacetyl production levels and the phenotypic and genotypic patterns observed. These strains suggest that spoilage lactobacilli can contribute to diacetyl production in wines.

Aroma Profiles of Research-Scale and Commercial New Zealand Pinot noir Wines

Sonal Maharaj, Marion Piquet, Parimal Patel, Laura Nicolau, Randy Weaver, and **Paul A. Kilmartin***
University of Auckland, Department of Chemistry, Wine Science Programme, Auckland, New Zealand [p.kilmartin@auckland.ac.nz]

Thirty research-scale wines, made from 12 kg lots of grapes from vineyards in the Marlborough, Central Otago, and Martinborough regions of NZ, along with 80 commercial wines from seven NZ regions, Burgundy, and Australia (2005 or 2006 vintages), were analyzed for a range of volatile aroma compounds. Various esters, terpenes, C6 alcohols, and higher alcohols were quantified from a 1:1 diethylether: hexane extract, while further extraction procedures used routinely in our laboratory in the study of NZ Sauvignon blanc wines were used for volatile thiols, such as 3-mercaptohexan-1-ol (3MH) and methoxypyrazines, with all extracts run on a GC-MS. A wide variety of esters and higher alcohols were determined in the wines and the concentrations were consistent with previously reported values. Several of the esters were present at higher levels in commercial wines from Burgundy and Australia in this survey, which may reflect the influence of warmer climates or particular growing seasons. In the research wines, the level of methoxypyrazines such as 2-methoxy-3-isobutyl pyrazine (IBMP) were quite low (typically <5 ng/L). On the other hand, the concentrations of the volatile thiol 3MH were in the range of 800–1000 ng/L in the research wines, which can potentially add grapefruit or blackcurrant type characters to the red wine.

Production and Characteristics of Wines Produced with Domestic Cultured Grapes in Korea

Jung-In Kim, Nam-Keun Lee, Byung-Wook Yang, Il-Jae Cho, Eun-Ju Yun, and Young-Tae Hahm*
Department of Biotechnology, Chung-Ang University, Anseong-Si, Gyeonggi-Do, 456-756, Korea [ythahm@cau.ac.kr]

Wines were produced with domestic cultured red grape varieties such as Campbell Early, Geubong, and Steuben. Single and blended grape juices were used to produce wines: Campbell Early 100% (C), Geubong 100% (G), Steuben 100% (S), Geubong 70%/Steuben 30% (GS), and Geubong 70%/Campbell Early 30% (GC). During fermentation, the sugar content of C rapidly decreased. After fermentation, the pH of each wine was between 3.25 and 3.50. Total acidities and total phenol contents of C, G, S, GS, and GC were 6.75, 6.07, 6.47, 6.60, and 7.23 g/L and 839, 808, 719, 824, and 812 mg/L, respectively. In color density analysis, redness(a*) values of C, G, S, GS, and GC were 29.34, 38.23, 37.10, 27.10, and 36.32, respectively. Wines were stored at 4°C for 40 days and then racking was carried out. After racking, pH values of C, G, S, GS, and GC wines were 3.23, 3.49, 3.55, 3.54, and 3.53 and total acidities were 6.63, 5.56, 6.03, 5.86, and 5.60 g/L. After 90 days stored in a cold room (4°C), the C, G, S, GS, and GC wines contained 1.9, 1.3, 1.2, 1.2, and 1.2 g/L of tartaric acid, 1.4, 1.5, 2.6, 2.3, and 2.2 g/L of malic acid, and 0.3, 0.5, 0.5, 0.5, and 0.5 g/L of lactic acid. The preferences of aroma and taste were evaluated

by quantitative descriptive analysis. Wines produced with Campbell Early received the highest score in sweet flavor and Geubong/Steuben was highest in fruity flavor. Steuben wine had high sweetness in taste and Geubong/Campbell Early wine had a highly acidic taste. Results indicated that domestic cultured grape varieties could be used to produce red wines.

Isolation and Characteristics of Wild Yeast from Domestic Cultured Grapes in Anseong, Korea

Jung-In Kim, Nam-Keun Lee, Byung-Wook Yang, Il-Jae Cho, Jung-Su Lee, and Young-Tae Hahm*
Department of Biotechnology, Chung-Ang University, Anseong-Si, Gyeonggi-Do, 456-756, Korea [ythahm@cau.ac.kr]

Wild yeasts suitable for wine production were isolated from the domestic cultured grapes in the region of Anseong, Korea. Ten strains were isolated from the naturally fermented grape juices of Geubong, Campbell Early, and Meuru varietals. The isolated strains were identified using 26S rDNA sequencing. Seven of 10 strains showed 99.9% similarity to *Saccharomyces cerevisiae*. Five strains were isolated from Geubong grapes and two strains from Campbell Early and Meuru grapes. The remainder were identified as *Hanseniaspora* spp. and *Candida humilis*. During fermentation at room temperature for 7 days, sugar contents decreased from 28.0 to 12.6 Brix fermented with one of the Gongdo strains and to 15.6 Brix with the Daerimdongsan strain. Results indicated that the strain isolated from Geubong grape in the Gongdo region had higher fermentative ability than the other strains. In the API assimilation test, the stain isolated from Geubong grapes in the Docjungri region was positive with all of 19 tested sugars, including glucose, glycerol, and D-raffinose. The strain from Daerimdongsan was also positive with most sugars, except 2-keto-D-gluconate, L-arabinose, adonitol, and inositol. The strains isolated from Campbell Early and Meuru grapes in the Shinchonri were positive with some sugars, including glucose, glycerol, D-saccharose, and D-raffinose. These results demonstrated that *Saccharomyces* spp. isolated from different areas and grape varietals have slightly different biochemical activities in fermentative and sugar assimilation abilities.

Relationship between H_2S Production and MET Genes Expression Levels in *S. cerevisiae* under Different Assimilable Nitrogen Status

Ana Mendes-Ferreira, Catarina Barbosa, Arlete Mendes-Faia,* and Cecília Leão
IBB-Centro de Genética e Biotecnologia, Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal [afaia@utad.pt]

Nitrogen deficiency has been associated with serious problems encountered in contemporary winemaking, particularly slow and/or incomplete fermentations and hydrogen sulfide (H_2S) formation. Hence, we followed the time course of H_2S production in synthetic grape-juice media mimicking the natural environment of winemaking conditions, using an experiment design based on the conditions formerly established in our laboratory. For these studies, we used an average sulfide producer, *Saccharomyces cerevisiae* PYCC 4072, in which genomewide expression analysis had been undertaken under different nitrogen regimes. In this context, an integration

of expression data of genes involved in sulfur assimilation and sulfur amino acid biosynthesis with hydrogen sulfide production associated to nitrogen deficiency was performed. The lowest H_2S production was detected for the lowest nitrogen concentration used (66 mg N/L), where according to the literature one expected the highest H_2S production. Transcriptomic data obtained showed that the genes that are specifically down-regulated under conditions of nitrogen deficiency. The highest amounts of H_2S were observed in media with 267 mg N/L of initial nitrogen concentration. Nitrogen addition to the N-limiting fermentation led to an increase of all *MET* genes, anticipating H_2S liberation. These results question the common practice of nitrogen supplementation to overcome sulfide production.

Effect of Stainless-Steel Vats and Glass Carboys on Muscadine Wine Dissolved Oxygen, Color, Total Phenols, and Titratable Acidity

Mitwe Musingo* and Lihong Wang
Food Science Program and Center for Viticulture and Small Fruits, Florida A&M University, Tallahassee, FL 32307 [mitwe.musingo@famu.edu]

The aim of this study was to compare the effect of stainless-steel vats with glass carboys on red muscadine (*Vitis rotundifolia* cv. Noble*)* dissolved oxygen, color, total phenols, and titratable acidity. It was hypothesized that stainless-steel vats, which could introduce a micro amount of oxygen into wine, thus allowing microoxygenation to occur, would enhance the stability of muscadine wine. The grapes were fermented in two carboys (5 gallons in size) and two stainless-steel vats (7 gallons in size) for 18 months, and wine samples were analyzed every two weeks to determine the oxygen level, color, total phenols, and titratable acidity. Results showed that oxygen levels in wine from stainless-steel vats were continuously higher than in wine from glass carboys. A higher color hue started after three months of fermentation in stainless-steel vats and this trend continued for the 18 months storage time. Total phenol values were not significantly different in the two wines. Titratable acidity values were lower in wine from the stainless-steel vats than wines from glass carboys.

Role of Primary Fermentation Conditions in the Formation of Sulfur Taints Sur Lie

Laura Oskwarek, Yeun Hong, and Linda F. Bisson*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [lfbisson@ucdavis.edu]

Sur lie aging contributes many positive characteristics to wine; however, sulfur containing off odors (sulfur taints) may also be produced during sur lie aging and negatively impact wine perception. Previous research has suggested a correlation between hydrogen sulfide formation during the primary fermentation and the formation of sulfur taints sur lie. This study investigated the role of sulfide formation during primary fermentation and several primary fermentation factors including: nitrogen level, vitamin level, combination of vitamin and nitrogen limitation, level of methionine and cysteine, and level of sulfate. Fermentations were carried out using five different strains displaying distinct behaviors in the formation of sulfide

in both synthetic media of defined composition and in grape juices. Three strains, UCD522, UCD940, and UCD950, produced moderate to high levels of H_2S in all cases. Fermentations were aged sur lie for up to seven weeks. At the end of aging, samples were analyzed by GC for sulfur taint compounds. In Chardonnay wine, strains UCD522 and UCD950 produced similar amounts of H_2S following primary fermentation but only UCD950 had remaining H_2S four weeks sur lie. Additional sulfur taints above threshold were also detected for UCD950 four weeks sur lie. There was no correlation between the amount of H_2S produced and the timing of production of the H_2S in relation to the appearance of sulfur taint compounds. The sulfur taint compounds formed were generally present in levels below their sensory threshold.

Assessment of Perceived Wine Astringency by Cyclic Voltammetry

Steven C. Petrovic*
Department of Chemistry, Southern Oregon University, Ashland, OR 97520 [petrovis@sou.edu]

Cyclic voltammetry (CV) has been investigated as an analytical method in assessing perceived wine astringency. Because tannins are the major source of phenolics in red wine and play a critical role in red wine astringency, it was anticipated that the CV response of tannins would provide a significant level of correlation with wine astringency. A set of 35 red wines that had previously undergone sensory evaluation were each diluted in a model wine matrix and their CV responses were obtained at a glassy carbon electrode. Using a 200-fold dilution of the original wine sample, the current at each significant CV peak (approximately -460 mV, -660 mV, and -860 mV (vs Ag/AgCl)) was moderately to significantly correlated (R^2 = 0.50 to 0.68) with the perceived astringency. However, the peak current ratio between -860 mV and -660 mV exhibited a significant negative correlation with perceived astingency (R^2 = 0.77), suggesting that electrode passivation due to the presence of pyrogallol functional groups may serve a role in astringency assessment. Under dilute conditions, passivation is seen in the CV response of monomeric flavan-3-ols possessing a pyrogallol functional group. These results indicate that cyclic voltammetry may be a useful and convenient analytical method for the assessment of wine astringency.

Effect of Metal Chelators on Wine Oxidation

Annegret Rust, Anne-Laure Boivin, Ryan J. Elias, and Andrew L. Waterhouse*
Department of Viticulture and Enology, University of California, Davis, CA 95616
[alwaterhouse@ucdavis.edu]

The control of chemical oxidation is of critical importance with respect to wine quality, yet many of the mechanisms governing this process are still poorly understood. Recent studies in our laboratory and elsewhere have demonstrated the importance of endogenous transition metal catalysts in the course of wine oxidation. In this study, we examined the effect of metal chelators on the suppression or acceleration of wine oxidation. Ethanol oxidation, as measured by acetaldehyde formation, as well as quinone formation was measured to assess the extent of oxidation. Catalytic concentrations of iron were added to wine and model wine solution, as well as various metal complexing agents, including ethylenediaminetetraacetic acid (EDTA), defer-

oxamine, and phytic acid. The ability of these chelators to disrupt redox cycling of transition metals has been widely demonstrated in many systems, yet never in wine. Here we report the effect of these chelators on acetaldehyde production in wine systems under aerial and anoxic conditions, and in doing so demonstrate the importance of trace metals in the overall scheme of wine oxidation. This study provides a better mechanistic understanding of wine oxidation, with the goal of yielding new tools for the control of wine oxidation.

Chemical Basis for Wine Body: Exploratory Study of GC-MS and NMR Metabolite Identification in White Wines

Kirsten Skogerson,* Ron Runnebaum, Hildegarde Heymann, and Oliver Fiehn
Genome Center, University of California, Davis, CA 95616 [skogerson@ucdavis.edu]

One key attribute of any wine is its body, or viscous mouthfeel properties; despite the importance of body on the style and quality of wine, its precise origins remain unclear. Using a chemometric approach, metabolite profiles in conjunction with sensory assessments allow for the identification of individual chemical compounds correlated with sensory characteristics of a wine. Ultimately once compounds are identified, viticultural and enological practices that influence their concentrations can be elucidated and subsequently implemented to target the desired sensory characteristics. This study compares gas chromatography-coupled mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR)-based metabolite profiles in the characterization of white wines including Chardonnay, Viognier, Pinot Gris, Riesling, and Sauvignon blanc. The goal was to identify compositional differences in these wines that correlate to wine mouthfeel properties. The metabolite profiles of 17 white wines were determined independently using GC-MS and ^{1}H-NMR. Over 300 metabolites are reliably detected in all wine samples by GC-MS and over 100 of these are uniquely identified by retention index-based mass spectral libraries. One-dimensional NMR data yields far fewer metabolites (<50) and this list comprises a subset of those metabolites detected by GC-MS. Correlations were identified between the sensory data, which was obtained using descriptive analysis techniques, and both sets of instrumental data. Partial least squares multivariate models were used to explain the mouthfeel viscosity rating of the 17 white wines. The independently calculated MS and NMR models present new insights into the chemical basis for wine mouthfeel properties.

Monitoring the Functionality of Yeast Cells Using Flow Cytometry

Stephan Sommer* and Karl-Josef Hutter
State Education and Research Center for Viticulture & Horticulture (DLR–Rheinpfalz), 67435 Neustadt/Weinstrasse, Germany [stephan.sommer@dlr.rlp.de]

The aim of this study was to identify parameters and to establish methods for viable yeast cells that help to assess the functionality of the metabolism and the vitality of the cell. Throughout the fermentation process, yeast is facing changing conditions and has to react by adapting its metabolism. The adaptation process is the critical point in every fermentation and is based on the accurate function of the cell. The membrane-potential, which is responsible for protecting the yeast from low

pH-values, plays an important role for evaluating functionality. Other direct indicators are storage products like glycogen and neutral lipids, as well as mitochondrial activity and the degree of DNA damage. These parameters are completed by the measurement of indirect products and the closer inspection of following reactions of the yeast cell like viability, cell cycle, intracellular pH value, and reactive oxygen species inside the cell. The correlation of these factors provides useful criteria for evaluating the performance and refractiveness of the yeast. In the present study, a *Saccharomyces cerevisiae* yeast was conducted in a must media for 384 hr (16 days) and analyzed for its viability and functionality parameters. Results show a clear confirmation of theoretical coherence. Furthermore it is possible to gain deeper insight in stress response and adaptation behavior of yeast. The causality from the formation of oxygen-radicals to necrosis or apoptosis could be proved metrologically, as well as the dependency of the intracellular pH value on the stability of the membrane. This biomonitoring system using flow cytometry provides valuable information as a time-saving process control for wineries.

Calcium Tartrate Stabilization in Wine with Phytic Acid

Brent C. Trela*
Texas Tech University, Department of Plant and Soil Science, Lubbock, TX 79409 [brent.trela@ttu.edu]

An alternative method of calcium tartrate (CaT) stabilization for a model wine was investigated at laboratory scale using phytic acid (PA), a form of phosphorus storage in plants. Treatments with decreasing doses of PA at concentrations ranging from PA:Ca molar ratios of 1:1 to 1:10 prevented CaT precipitation in all samples to which it was added and may be effective at lower concentrations than tested in this study. The control model solution without PA showed significant visible precipitation after just two days at room temperature, while none of the samples containing phytic acid showed any precipitation after 1 month, indicating that PA is effective at keeping CaT soluble. Phytic acid treatment did not produce precipitates or result in significant reductions of Ca in a model wine. However, lower PA to higher Ca treatment ratios need to be investigated, as well as determining the effects on both the sensory perception and the long-term stability of wines treated with PA under actual winemaking conditions.

Evaluating Egg White Fining as a Means to Reduce *Brettanomyces* Populations in Merlot Wine

Jennifer Walsh, C.M. Lucy Joseph, and Linda Bisson*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [lfbisson@ucdavis.edu]

The yeast *Brettanomyces bruxellensis* produces several metabolites which contribute to spoilage taints in wines. While sterile filtration and chemical treatment in wine can control growth, winemakers may prefer to avoid these practices for a variety of reasons. This study evaluated the ability of egg white fining and biotin elimination to reduce *Brettanomyces* in wine. Prior research indicates that biotin and thiamin are the only vitamins required by all *Brettanomyces* strains tested. In addition to poten-

tial inhibitory proteins, egg whites also contain the glycoprotein avidin, which binds biotin and may render it unavailable for microbial metabolism. Initial tests indicate that using avidin as a fining agent depletes levels of biotin in *Brettanomyces* cultures and that this depletion has a negative impact on growth. Further fining trials with Oakville Merlot wine using powdered and fresh egg whites as a source for avidin are in progress.

Development of Research-Scale Winemaking Procedures to Represent Grape Quality in New Zealand Pinot noir

Sonal Maharaj, Marion Piquet, Paul A. Kilmartin, Laura Nicolau, and **Randy Weaver***
University of Auckland, Department of Chemistry, Wine Science Programme, Auckland, New Zealand [r.weaver@auckland.ac.nz]

During the 2007 vintage, small batches of wine were made from Pinot noir grapes harvested from three New Zealand viticultural regions: Marlborough, Central Otago, and Martinborough at the same target ripeness (24 Brix). Three vineyards were harvested in each region and triplicate samples (~12 kg each) were taken from each vineyard in each region to give a total of 27 wines produced. The aim was to develop standardized research-scale winemaking techniques to produce wines that consistently represented the grapes from which they were produced. Standard chemical analyses including total acidity, volatile acidity, pH, reducing sugar, alcohol, and malic acid levels were performed on the wines. In addition, color properties were measured, giving information about anthocyanin content and SO_2-resistant pigment. HPLC measurements of monomeric polyphenols and levels of tannin using the methyl cellulose precipitation assay were also determined. Results showed consistency among the triplicate samples, which gave confidence that the minimal-intervention winemaking procedures had represented the grapes well. The analyses were used for a comparison between vineyards and also between grapegrowing regions. Color levels were consistent within regions but the wines made from grapes from the three Martinborough vineyards produced wines with higher levels of color and phenolics in this particular study. The analyses of the research wines were also compared to the analyses of 80 commercial wines from seven New Zealand regions and from Burgundy and Australia. The research wines had color levels and phenolic compounds ~30% lower than the commercial wines, as expected given differences between the small-scale and commercial pressing operations.

Color Development in Red Wine with a Low Tannin to Anthocyanin Ratio

Karl L. Wilker*
Department of Agriculture, Missouri State University, Mountain Grove, MO 65711 [KarlWilker@missouristate.edu]

The ratio of tannin to anthocyanin is considered an important variable in the development of red wine color. Optimum color development is thought to occur when tannin is present at a higher molar concentration than anthocyanin. Wines with less tannin than anthocyanin are thought to have poor color stability due to insufficient formation of anthocyanin-tannin polymers. An experiment was conducted to de-

termine the effect of tannin additions on polymeric pigment formation and color stability in a wine initially low in tannin and high in anthocyanin. Commercial grape seed tannin was added at 500 and 1000 mg/L levels to Norton wine after pressing and settling. Norton wine without the addition of tannin was used as a control. Each treatment replicate started initially as 15 gallons of must. The wines were periodically splash racked to incorporate oxygen during aging. The tannin additions resulted in higher levels of total and large polymeric pigments and lower levels of small polymeric pigments (14 months after crush). The treatments did not result in significant differences in color (absorbance at 420, 520, and 620 nm) between the wines. Small and large polymeric pigments continued to increase in concentration in all of the treatment wines as they aged. The ratio of tannin to anthocyanin seemed to have minimal impact on color during the first year of aging these wines.

Wine Closure on Volatile Sulfur and Aroma Development during Postbottle Aging

Xue Zhang and Michael Qian*
Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331
[michael.qian@oregonstate.edu]

It is generally accepted that bottled wine is a dynamic system. Wine closure can have a dramatic impact on wine postbottling behavior. It was found that wine developed differently based on the type of closure. In addition to providing the proper seals, wine closure should provide a proper gas exchange. The right amount of oxygen ingression to the wine is important to the formation of aged wine aroma. Too much oxygen ingression from the closure could oxidize the wine, while too little oxygen ingression, as happened in screwcapped wines, could induce reduced sulfur off-flavor formation. It is proposed that this off-flavor formation is due to the equilibrium shift of dimethyl disulfide to more powerful methanethiol at low redox potential. Chardonnay and Pinot noir wines were bottled at Argyle winery. Three types of commercial screwcap closures (Saran-tin, a Saranex, and a polyethylene) with low, medium, and high oxygen permeability were used. In addition, one plastic closure and one commercial natural cork (up and inverted) were compared with the screwcaps. Headspace oxygen was measured by Oxysense 4000B and Oxypress with puncturing needle. Dissolved oxygen was measured with an Orbisphere 3650. Volatile sulfur profile (MeSH, EtSH, H2S, DMS, DMDS, DMTS, DES, DEDS, MeSOAc, EtSOAc) was analyzed using solid phase microextraction-gas chromatography-pulsed flame photometric detection.

Effects of Abscisic Acid on Phenolic Composition of Cabernet Sauvignon and Merlot Winegrapes

Mauri Anderson, Matthew Fidelibus, and Andrew Waterhouse*
Department of Viticulture and Enology, University of California, Davis, CA 95616
[alwaterhouse@ucdavis.edu]

Abscisic acid (ABA) was applied at two concentrations (200 and 400 mg L^{-1}) and at two phenologically based events (veraison and veraison plus 4 weeks) to Cabernet Sauvignon and Merlot vines in Madera County during the 2007 growing season. This study evaluated the effects of these treatments on dermal phenolic content through the ripening period to harvest. Both cultivars responded to the application of ABA with increases in several phenolic compounds. Additionally, these responses were influenced both by timing and concentration of the application. Application of both 200 and 400 mg L^{-1} ABA at veraison to Cabernet Sauvignon resulted in statistically significant increases of several phenolic components including flavon-3-ols and flavones, as well as color density, hue, and visible color at harvest, when compared with control fruit. These effects were first seen two weeks after the veraison application and exhibited a dose-response, being greater for the 400 mg L^{-1} treatment. The postveraison treatment to Cabernet Sauvignon also increased color density, flavone absorbance, and visual color at both ABA concentrations, although to a lesser degree than the earlier application. Application of ABA to Merlot showed mixed results. Based on this study, we expect wine produced from ABA treated grapes to show modest increases in color and certain phenolic components. Future studies will be needed to confirm these effects and their magnitude.

Datalogging Harvest Snips Make Easy Work of Cluster Counting

Mark C. Battany*
University of California Cooperative Extension, San Luis Obispo, CA 93401
[mcbattany@ucdavis.edu]

The number of grape clusters per vine or per vineyard block is one of the most fundamental parameters used to predict, adjust, and evaluate crop yields. However, the common methods for counting clusters, either through simple counting or with a manual hand counter, are generally regarded as being inefficient and very often inaccurate, particularly when the individual performing the task is fatigued or distracted. To improve both the efficiency and accuracy in making field cluster counts, datalogging harvest snips were developed that automatically record each open/close cycle of the snips, and hence the number of clusters harvested by an individual using the snips. The devices consist of a standard pair of harvest snips, with a magnetically activated reed switch and miniature datalogger attached to one handle, and a magnet attached to the opposite handle; each open/close cycle of the snips is recorded as a unique time event on the datalogger. Ten identical devices were then assembled for harvesting three large winegrape field trials in fall 2007. Harvests were conducted by two different commercial labor crews, which were both trained beforehand in the proper use of the snips. This produced comprehensive records of the total number of clusters harvested in each individual research plot, acquired with very little additional effort beyond tabulating the output of the dataloggers later that same day.

These types of devices could be very useful in both research and commercial vineyard activities for acquiring accurate and reliable cluster count information with relatively little effort.

Allelic Relationship of Root-Knot Nematode Resistance Genes in a *Vitis nesbittiana* Population

Peter Cousins* and Laurie Boyden
U.S. Department of Agriculture–Agricultural Research Service, Grape Genetics Research Unit, Geneva, NY 14456 [peter.cousins@ars.usda.gov]

Root-knot nematodes, *Meloidogyne* spp., are a major pest of grapes worldwide. Populations of *Meloidogyne* have been isolated that are capable of feeding and reproducing in the presence of the *N* allele, the principal source of genetic resistance to root-knot nematodes in grape. An accession of *Vitis nesbittiana* resistant to *N*-virulent nematode strains was identified, and progeny testing determined that the accession is heterozygous for a single dominant resistance allele with specificity different from that of *N*. We hypothesized that the new allele is found at a distinct resistance locus and is not an allele of the N gene. The relationship of the novel allele, tentatively *R,* to *N* was determined using progeny testing. The resistant accession (genotype nnRr) was crossed with the rootstocks Freedom, homozygous for the *N* allele (NNrr). Progeny from the Freedom x *V. nesbittiana* cross were screened with *N*-virulent nematodes and resistant seedlings (putative genotype NnRr) were selected. Pollen was collected from flowering resistant seedlings and used to make test crosses with a nematode-susceptible female, the rootstock 161-49C (nnrr). Test cross progeny (NnRr x nnrr) were screened with *N*-avirulent nematodes and segregated 3:1 for resistance, indicating that N and R are nonallelic and represent two distinct resistance loci. This knowledge will facilitate the use of the resistant *V. nesbittiana* accession in breeding to produce new rootstock cultivars resistant to *N*-virulent nematodes.

Effects of Cover-Crop Management on Vine Development and Fruit Quality of Pinot noir

Levi Fredrikson and Patty Skinkis*
Oregon State University, Department of Horticulture, Corvallis, OR 97331 [skinkisp@hort.oregonstate.edu]

Three cover-crop management treatments—complete tillage (C), alternate row tillage (A), and solid cover (C)—of an established grass mix cover crop were evaluated at two commercial Pinot noir vineyard sites (Archery Summit and Stoller) in the North Willamette Valley, Oregon, during 2007. Effects of irrigation (dry vs. irrigated) were further evaluated at Stoller. Vine growth parameters measured were not significantly impacted by cover-crop management, resulting in no treatment or irrigation differences. Soil moisture differed with cover-crop management treatments at the 46 cm depth throughout the entire season, with A having the lowest volumetric soil moisture compared with C and S. Winegrape parameters such as berry weight, degrees Brix, pH, and titratable acidity were evaluated during the ripening period up to harvest, and differences were not found for cover-crop management treatments,

although degrees Brix of dry treatments were higher than irrigated treatments (p = 0.02). Three measures of grape quality important to wine production (yeast assimilable nitrogen content, skin polyphenols, and anthocyanins) were measured postharvest. YANC did not differ in fruit analyzed from different cover-crop management or irrigation treatments. Treatment C at the Archery Summit site yielded higher total berry skin polyphenols than A (p = 0.04), while treatment S at the Stoller site yielded higher total berry skin polyphenols than C (p = 0.05), with no differences in skin polyphenols seen across irrigation treatments. Total berry skin anthocyanin concentration followed a trend in which increasing vineyard floor cover (S>A>C) corresponded to increased anthocyanin levels.

Efficacy of ABA Application to Enhance Fruit Color of Cabernet Sauvignon Grape in a Warmer Growing Region

Sanliang Gu,* Sean Jacobs, Guoqiang Du, Xueqiang Guan, and Robert Wample
Viticulture and Enology Research Center, California State University, Fresno, CA 93740 [sanliang@csufresno.edu]

It is well established that abscisic acid (ABA) application improves fruit color development in grapevines. Experiments were conducted for two years to investigate the efficacy of and develop protocol for ABA application to enhance fruit color of Cabernet Sauvignon in the San Joaquin Valley, California, a warmer region for winegrape production. To identify the most effective ABA concentration and timing, clusters of mature Cabernet Sauvignon were sprayed with ABA at 300 or 600 ppm at the beginning of berry softening or coloring in 2006, as well as at 600 ppm at 1, 30, and/or 80% berry coloring in 2007. To determine the proper ABA application method, only leaves were treated with ABA at 600 ppm or only one cluster per shoot was treated while the other cluster on the same shoot was left untreated. ABA treatments were effective in enhancing skin anthocyanin content and fruit color in both years. Some ABA treatments increased skin anthocyanin content by ~100%. ABA applied during fruit coloring at 600 ppm was more effective than when applied earlier at 300 ppm in 2006. In 2007, double ABA applications at 1+80% or 30+80% berry coloring were more effective than a single application at 30% berry coloring. ABA applied only to leaves or adjacent clusters did not enhance skin anthocyanin content of the untreated clusters. For maximum color enhancement of Cabernet Sauvignon in a warmer growing region, ABA should be applied directly to the fruit at 600 ppm twice during 30-80% berry coloring.

Flavonols in Red and White Grapes Arising from Intraspecific Crosses from Monastrell x Syrah

Alberto Hernández-Jiménez, Rocío Gil-Muñoz, José M. López-Roca, and Encarna Gómez-Plaza*
Universidad de Murcia, Department of Tecnología de los Alimentos, Nutrición y Bromatología, 30100 Murcia, Spain [encarnag@um.es]

Flavonols are important flavonoids in grapes since they are implicated in red wine color (as copigments) and associated with the healthy properties of wines (as antioxidants). The concentration of these compounds in grapes has been shown to be

highly dependent on light exposure of the clusters, while their qualitative profile is genetically determined and, therefore, stable into each grape variety. Monastrell (Mourvedre, Mataro) is a variety very well adapted to the agroecological conditions of southeast Spain, but there are some problems with the color stability of its wines. The study of the grapes obtained from intraspecific crosses with Syrah could be interesting in order to select those seedlings showing the most favorable viticultural and enological characteristics of both varieties. We have studied the concentration and the flavonol profile through a collection of 39 red and white grapes arising from intraspecific crosses from Monastrell and Syrah by HPLC. The plants obtained from these intraspecific crosses were cultivated in the same location and the vines and grapes undergo the same agroecological conditions. Results show differences between the profile of red and white grapes and with their parentals. In general, white grapes show lower values of total flavonols than red grapes and absence of trihydroxilated flavonols. These facts could suggest that behind the white grape character, several enzymatic expressions and/or actions are being modified in the phenylpropanoid pathway, expressions not only involving the inhibition of anthocyanin biosynthesis, but also changes in the flavonol biosynthesis pathway.

High-Density, Multiple Depth, Wireless Soil Moisture Tension Measurements for Irrigation Management

Mark Holler*
Camalie Vineyards, Napa, CA 94558 [mholler@pacbell.net]

Soil moisture tensions from resistance measurements of granular matrix devices with gypsum solute source were compared to measurements of midday leaf water potentials to determine if the less labor intensive soil moisture tensions could substitute for or augment leaf water potential measurements most often used in regulated deficit irrigation management of grapevines. When sampled sufficiently at appropriate depths soil moisture tensions were found to correlate well with pressure chamber measurements of midday leaf water potential in Cabernet Sauvignon grapevines. Sampling two to three sites per acre across a 4.4 acre hillside vineyard during the 2007 growing season produced a substantial correlation of midday leaf water potentials to soil moisture tensions at 61 cm depth; $R^2 = 0.42$. No correlation with soil moisture tension measured at 30.5 cm depth was observed. Soils in the vineyard monitored are predominantly clay with a gradient of organic materials diffusing across the vineyard from an uphill forest watershed. Watermark granular matrix soil moisture tensiometers were used to measure the soil moisture tensions. The data suggests that soil moisture tension measurements may be able to replace many leaf water potential measurements, which are significantly more labor intensive. A strategy for use of soil moisture tension measurements in managing regulated deficit irrigation of grapevines is presented which does not require ET calculations. The self-organizing wireless sensor network used to aggregate the soil moisture tensions and serve them on the internet in real time is briefly described.

Clonal Rootstock Interaction in Syrah Grapevines

Bruce Jordan and Keith Patterson*
Department of Horticulture and Crop Science, California Polytechnic State University, San Luis Obispo, CA 93401 [kpatters@calpoly.edu]

The quality and the wine composition of grapes are influenced by the interaction between rootstocks and scions of grapevines. Over a growing season, the rootstocks evaluated were Millardet et de Grasset 420A (420A), 5C Teleki (5C), Millardet et de Grasset 101-14 (101-14), and Schwarzmann grafted onto clones of *Vitis vinifera* L. cv. Syrah 01, 174, 383, and 470 in San Luis Obispo, CA. After veraison, cluster weights and berries per cluster were measured to factor the crop yields and quality at harvest. The berries were analyzed for pH, soluble solids, and titratable acidity weekly until harvest followed by wine production of each rootstock/clone combination. Wine analysis will be performed by a sensory panel for flavor and aroma compounds present in the wine. The berry analysis of each rootstock/clone combination showed little variation between berry weight, pH, or soluble solids; however, the concentration of tartaric acid had significant variation between rootstocks. The total phenolics, including color intensity, color hue, and anthocyanins in the berries, also presented differences among rootstocks. The rootstock selection of a grapevine and their interaction with clones of Syrah may influence the fruit phenolic compounds and tartaric acid levels found at harvest for wine production.

Gas Exchange Response of Field-Grown Merlot Grapevines as Influenced by Rootstock

Michael Krawitzky, Thayne Montague,* and Ed Hellman
Department of Plant and Soil Science, Texas Tech University, Lubbock, TX 79409 [thayne.montague@ttu.edu]

In west Texas there is a great need to evaluate rootstocks for adaptability to the semiarid climate. The objective of this experiment was to determine gas exchange response of Merlot grapevines grafted to four different rootstocks (110R, 1103, 44-53, and 5BB) in a semiarid climate. The experimental design was a completely randomized block with five blocks and five plants of each rootstock/scion combination within each block. For each rootstock/scion combination there were a total of 25 plants. During 2007 weekly midday leaf water potential, stomatal conductance, and leaf temperature were measured. Mean daily water potential data indicate few differences in water potential between rootstock/scion combinations. Mean water potential for the entire measurement period indicates no rootstock differences. Rootstock/scion differences for mean daily stomatal conductance were found on several occasions. However, means for the entire measurement period indicate no differences between rootstock/scion combinations. Linear regression revealed stomatal response to leaf to air vapor pressure difference did not differ between rootstock/scion combinations. Despite descriptions of adaptability to drought conditions, our data indicate that during the experimental period overall differences in gas exchange between rootstock/scion combinations were not found. To further assist growers in west Texas and other semiarid climates, additional rootstock/scion research should be conducted.

Effect of Ethephon on Berry Splitting of the Grape (*Vitis vinifera* L.) cv. Flame Seedless

Gerardo Martínez-Díaz* and Arnulfo Márquez-Cervantes
Instituto Nacional de Investigaciones Forestales y Agtopecuarias, Hermosillo, Sonora, México
[germadz@hotmail.com]

Berry splitting causes severe losses in some years in the viticulture of Sonora, México. Berry splitting has been associated with the application of ethephon, as it occurs normally after the application of that hormone to the grape cv. Flame Seedless. The objective of this work was to determine the effect of ethephon and the adyuvant Latron on berry splitting of cv. Flame Seedless. In 2004 the two factors, the rate of ethephon and method of application, were evaluated. The rates of ethephon were 0, 0.5, and 1 kg i.a. ha^{-1}, and the methods of application were spraying to the clusters and foliage and spraying only to the foliage. In 2005 the rates of ethephon and rates of Latron B1956 were tested. The rates of ethephon were 0.5 and 1 kg i.a. ha^{-1} and the rates of Latron B1956 were 0.3, 0.6 and 0.9 lt PC ha^{-1}. In both years the treatments were distributed in a completely randomized block design with three replications and the experimental unit was a grapevine row with seven plants each in 2004 and with nine plants each in 2005. Berry splitting was higher in the treatment with ethephon at 0.5 kg i.a. ha^{-1} (>40 split berries in 100 clusters) than in the control (6.6 split berries in 100 clusters) ($p < 0.5$). The method of application of ethephon and Latron did not promote berry splitting. In 2005 there were four times more split berries than in 2004 at the beginning of the harvest and this number increased dramatically to 18 times one week later. In both years the increase of the rate of ethephon from 0.5 to 1 kg i.a. ha^{-1} did not increase the number of split berries.

Effect of Ethephon, Ethanol, and Potassium on Soluble Solids Accumulation in the Grape cv. Sugraone

Gerardo Martínez-Díaz* and Arnulfo Márquez-Cervantes
Instituto Nacional de Investigaciones Forestales y Agtopecuarias, Hermosillo, Sonora, México
[germadz@hotmail.com]

The accumulation of soluble solids in the grape cv. Sugraone is one of the problems in the viticulture of Sonora, México. Therefore the aim of this work was to investigate the effect of ethephon, ethanol, and potassium on the accumulation of soluble solids in that cultivar. The research was conducted in 2004, 2005, and 2006. In 2004 the ethephon at the rates of 0.25 and 0.5 kg de i.a. ha^{-1} and ethanol at the rates of 5 and 10 L ha^{-1} were compared to a control. In 2005 the treatments were: two applications of ethephon at 0.5 kg i.a. ha^{-1}, three applications of ethephon at 0.5 kg de i.a. ha^{-1}, two applications of ethanol al 10 L ha^{-1}, three applications of ethanol at 10 L ha^{-1}, and the control. In 2006 the ethephon at 0.5 kg i.a. ha^{-1} and ethanol at 10 L ha^{-1} were tested alone or combined with Codabrix at 4 lt/ha. Codabrix contains 25.3% K_2O plus 16.2% polysaccharide. The first application was made when the berries had 11 to 12 Brix in the three years. The treatments were distributed in a completely randomized block design with three replications and the experimental unit contained three grapevines in 2004 and 10 grapevines in 2005 and 2006. The content of solu-

ble solids was measured three times until harvest in one berry located in the central sector of each cluster in the grapevines. Ethephon, ethanol, and Codabrix did not cause any change in the soluble solids content of the berries.

Creating Canopy Exposure Maps Using New Metrics for Point Quadrat Analyses

James M. Meyers* and Justine E. Vanden Heuvel
Cornell University, Field of Horticulture, Ithaca, NY 14853 [jmm533@cornell.edu]

Optimal cultural practices require a thorough understanding of canopy microclimate, guided by precise biomass and sunlight distribution measurements. Point quadrat analysis (PQA) is useful but limited in its spatial precision and lack of sunlight calibration. To address this, we designed field sampling and numerical analysis methods that combine traditional PQA and sunlight measurements into a calibrated canopy sunlight exposure map. New metrics include: occlusion layer number (OLN), which quantifies the shading contacts per insertion; cluster exposure layer (CEL) and leaf exposure layer (LEL), which quantify the shading layers between a contact and its nearest canopy boundary; canopy cluster symmetry (CCS), which measures the positional bias of clusters along the row; a canopy calibration coefficient (Ep1) which uses a single measurement at the center of the canopy to calibrate the overall light distribution; cluster exposure flux availability (CEFA) and leaf exposure flux availability (LEFA), which use the canopy calibration to quantify sunlight available to each contact; cluster exposure flux symmetry (CEFS) and leaf exposure flux symmetry (LEFS), which measure the balance of sunlight exposure across the row; and trellis contact symmetry (TCS), which measures canopy consistency around the intended trellis centerline. Applied to sample data from a canopy management study, these techniques revealed a detailed understanding of biomass distribution, light environment, and treatment efficacy. The simplicity and precision of calibrated PQA is an effective alternative to time-consuming coordinate-based canopy measurement techniques and to less robust methods (e.g., sunfleck analysis).

Amino Acid Levels and Phenolic Biosynthesis in Skin and Pulp of Grape Berries during Ripening

Jonas Mueller and Douglas O. Adams*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [doadams@ucdavis.edu]

Several amino acids (AA) besides phenylalanine play a key role in the synthesis of phenolic compounds. One objective of this research was to determine if skin and pulp of the same grape berries would show different compositions of free AA due to different metabolism taking place. Another objective was to look at possible correlations among ratios and levels of AA and phenolic compounds found in the skin. Merlot grapes from two different rootstocks (101-14 and 1103-Paulsen) were sampled at the Oakville Experimental station (Napa, CA) at three different times during ripening in 2007. Previous work had demonstrated that Merlot musts from the 101-14 rootstock showed only about half the available nitrogen in AA as musts obtained from 1103-P fruit. For each treatment (rootstock) and sample date,

24 berries were carefully dissected into seeds, pulp, and skin. Different extracts were prepared for AA analyses in pulp and skin and phenolic analyses in seed and skin, respectively. Principal component analyses showed large differences in the amino acid composition of skin and pulp, between rootstocks, and among sample dates. However, the ratios between skin and pulp levels, and between the rootstocks, stayed constant among sample dates for most of the AA. The anthocyanin and tannin levels changed as expected during ripening but did not differ significantly between the two rootstocks at any sample date. These results demonstrate that different metabolism in pulp and skin lead to different AA profiles. However, no correlation could be established between level of AA and amount of phenolic compounds produced.

Influence of Temperature on Berry Composition of an Interspecific Hybrid Grape

Puspa Raj Poudel,* Kenji Beppu, Ikuo Kataoka, and Ryosuke Mochioka
Kagawa University, Faculty of Agriculture, University Farm, Sanuki, Kagawa 769-2304, Japan
[poudelpuspa@yahoo.com]

High temperature affects berry composition, especially titratable acidity, total soluble solids, and anthocyanin content. Because *Vitis ficifolia* var. *ganebu*, a wild grape of subtropical origin with a low chilling trait, develops good coloration in its natural habitat where daytime and nighttime temperatures are high during the berry ripening stage, it was postulated that hybridizing *V. ficifolia* var. *genebu* with *V. vinifera* would improve berry composition, particularly composition factors that affect berry coloration, at high temperature. The aim of this study was to investigate the influence of temperature on berry composition of Kadainou R-1 grape, an interspecific hybrid grape derived from *V. ficifolia* var. *ganebu* and *V. vinifera* cv. Muscat of Alexandria. Potted Kadainou R-1 vines (own-rooted) were subjected to continuous temperatures of 20, 25, and 30°C in a phytotron during the berry softening stage. Berries were harvested at 15, 30, and 37 days after the vines were placed in the phytotron. Titratable acidity was lower at 30°C than at 20°C while total soluble solids were highest at 25°C. Accumulation of both glucose and fructose was higher at low than at high temperature. Total anthocyanin content (mg/g^{-1} of fresh weight) was significantly reduced at 20 and 30°C but not at 25°C. Flavonols content was highest at 25°C.

Evaluation of Boron Fertilizer Applications to Pruning Wounds on Phytotoxicity

Rhonda J. Smith,* Philippe E. Rolshausen, and Walter D. Gubler
University of California Cooperative Extension, Sonoma County, Santa Rosa, CA 95403
[rhsmith@ucdavis.edu]

Eutypa lata causes grapevine trunk disease by infecting pruning wounds. In 2004, researchers reported that 5% boric acid (8750 mg/kg B) mixed in a commercial paste and applied to pruning wounds significantly reduced disease in field trials. Our objective was to evaluate the use of a commercial boron fertilizer applied to pruning wounds on phytotoxicity in field conditions. Trial 1 and 2 (2005) included rates of 1320, 6600, and 66000 mg/kg B. Trials 3 (2006) and 4 (2007) B rates were 1750 and 8750 mg/kg. Trial 5 (2007) included trial 4 rates plus a blank paste and

4380 mg/kg B. Each rate of B fertilizer was applied as a spray and mixed with a commercial paste. Treatments were applied to single vine replicates immediately after pruning and were artificially inoculated with *E. lata* ascospores. Percent budbreak was determined with the modified Eichorn-Lorenz system for each spur node position. Our results indicated that in trials 1 and 2, the greatest percent budbreak occurred in basal nodes after application of 66000 mg/kg B ($p = 0.0001$) and those treatments significantly reduced budbreak in the distal node ($p = 0.0000$). In trials 3 and 4, treatments did not affect budbreak. In trials 1 and 2, the 66000 mg/kg spray application resulted in higher mean B concentration in both petioles and blades at bloom but less consistently at veraison. In other trials there was no effect of treatment on B concentration in either tissue type or timing. No foliar boron phytotoxicity symptoms were observed.

Molecular Characterization of Bud Sport of Pinot Gris Bearing White Berries

Shunji Suzuki,* Terumi Furiya, Tamotsu Sueta, and Tsutomu Takayanagi
Laboratory of Fruit Genetic Engineering, The Institute of Enology and Viticulture, University of Yamanashi, Kofu, Yamanashi 400-0005, Japan [suzukis@yamanashi.ac.jp]

Bud sport is a somatic mutation in shoot cells of perennial fruits. We report here the molecular characterization of the white color mutation in Pinot Gris bearing white bunches. The Pinot Gris bud sport did not accumulate anthocyanins in the skin because of its inability to express a UDP glucose-flavonoid 3-o-glucosyl transferase transcript. Genomic analysis with cleaved amplified polymorphic sequence (CAPS) markers demonstrated that the bud sport is a periclinal chimera that is heterozygous with one red allele and three white alleles. However, the red allele has a large deletion in the color locus, resulting in white-skinned berries. In addition, the Pinot Gris bud sport showed triallelic profiles at the locus containing a microsatellite DNA marker, indicating that genetically different cell layers, L1 and L2, coexisted in the berries. Taken together, we conclude that the color mutation in Pinot Gris bud sport might have arisen from a large deletion in the color locus of the red allele of L1 cells, and not from L1 and L2 cell layer arrangements. These findings and further studies on Pinot Gris bud sport are expected to provide new information on the breeding systems of white grapevines as well as improve our understanding of the evolutional events in grapevine.

Three-Hour Winery and Field Test for *Brettanomyces*

Stewart Lebrun*
Z-Enology, Anaheim, CA 92807 [lebruns@uci.edu]

Brettanomyces species and substrains are a group of wine spoilage yeasts that produce metabolites associated with poor wine flavor and aroma. At high concentrations (over 10^4 cells/mL) modified phenolics and other undesirable metabolites produced by *Brettanomyces* build to levels that can substantially reduce wine quality. Current methods to test for *Brettanomyces* include selective plating, which takes over 10 days, and skill in microbiology and DNA based testing (PCR), which must be sent to an outside lab and is an expensive option for routine testing and may be prone to false positives. Recently we have developed and started to sell a rapid (3 hour) and inexpensive ($15/test) immunoassay test for *Brettanomyces*. The test is performed by applying a small amount of wine to a test chip, which is processed as an immunoassay with a semiquantitative result in 3 hours. The cell number is estimated by comparing the signal produced by the wine sample to be tested with a set of internal standards. Without concentration the test is sensitive to 10^4 cells/mL, and with a simple filtration step the sensitivity is 10 cells/mL. Recent improvements include the development of a decolorizing reagent and use of filtration to increase sensitivity to 10 cells/mL. The presentation will explain how the test works (specific steps and timing) as well as validation data and specific winery and field application results.

Enological Effects of Gum Arabic Resulting in Tartrate and Aromatic Stabilization

Marzio Mannino* and Gianni Triulzi
ENARTIS- ESSECO GROUP, San Martino di Trecate Novara 28069, Italy [marzio.mannino@enartis.it]

Can gum arabic help in tartrate and aromatic stabilization? We researched the action of gum arabic in relation to improving tartrate and aromatic stabilization. Gum arabic interacts with wine compounds in a manner consistent with its hydrocolloid nature, with positive results relating to the organoleptic properties of the wine. It is used because of its texturizing, film-forming, and emulsifying capacity. Such effects are related to its molecular structure, hydrophilic carbohydrates, and hydrophobic protein groups in such a manner as to produce polar, nonpolar, or Van der Waals bonds. Gum arabic can react with wine components via two mechanisms: (1) formation of "chemical bonds" with various organic molecules of various degrees of polarity (primarily aromas and organic acids), and (2) influencing the velocity of "mass transfer." In particular, the reduction of the speed of transfer of organic molecules from liquid to vapor, and then to the olfactory apparatus, for example, would be aroma compounds. These two phenomena work together in a nonpredictable manner as they cause a positive, negative, or null sensory response depending on factors tied to the composition of the wine and the physiochemical characteristics of the gum arabic used. In conclusion, the effects of gum arabic on tartrate and aromatic stabilization are important in the prebottling and the bottling phases. Results show increased shelf-life of the wine by preventing precipitation and preserving the original organoleptic properties.

Irrigation Water Stress Management: Study of Vineyard Transpiration with a Sap Flow Meter

Thibaut Scholasch,* Eric Lebon, Anne Pellegrino, and Jeremie Lecoeur
Fruition Sciences, Berkeley, CA 94704 [thibaut@fruitionsciences.com]

Water balance models on nonirrigated vineyards are commonplace. Those models rely upon a correct estimate of plant transpiration rate. However, the transfer of existing models to irrigated vineyards under high evaporative demand is difficult. Irrigation practices during high vapor pressure deficit (VPD) have contrary and complex effects on vine transpiration. Monitoring vine sap-flow allows a direct assessment of vine transpiration rate and offers improved understanding of the effects that soil moisture gradients and VPD have on the plant's water deficit. Current vine-water status assessments are based on discontinuous, difficult, and time-consuming leaf water potential measurements. Sap-flow measurements available now provide the vineyard manager with a continuous estimate of vine transpiration throughout the season. Assessing sap flow variations can indicate plant water status and provide a tool to optimize irrigation. We compare vine transpiration with stem water potential and soil moisture while explaining the advantages and inconveniences of this new method.

* indicates corresponding author

Using Marker-Assisted Selection to Breed for Pierce's Disease Resistance in Grape

Summaira Riaz, Alan C. Tenscher, Rachel Graziani, and **M. Andrew Walker***
Department of Viticulture and Enology, University of California, Davis, CA 95616 [awalker@ucdavis.edu]

Pierce's disease (PD), caused by the bacterium *Xylella fastidiosa,* is an important disease of grapevines in California and limits the cultivation of grapes across the southern United States. In regions where disease pressure is high, the cultivation of highly desired *Vitis vinifera* cultivars is difficult or impossible. This study reports on the introgression of PD resistance from forms of *V. arizonica* into elite *V. vinifera* wine, table, and raisin grape genetic backgrounds, and on the reliability of PCR-based marker-assisted selection (MAS) to accelerate the breeding of PD-resistant grapes. A total of 3,967 genotypes (F1, BC1, and BC2) were screened with two markers (VVIP26 and ctg1026876) in spring 2006 and 2007. Spring 2008 will focus on BC2 and BC3 progeny. Resistance alleles for both markers, which were unique in size and not shared by susceptible *V. vinifera* selections, were present in 1,527 seedlings. The distinctiveness of these alleles and their inheritance as single dominant gene has allowed MAS to optimize and greatly expedite the breeding of PD-resistant grape cultivars. Fruit and wine quality data from advanced selections with 87.5% *V. vinifera* will also be presented.

Effects of Climate and Nitrogen Nutrition on the Activation and Spread of Latent Botrytis Infections

Stella M. Zitter and **Wayne F. Wilcox***
Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456 [wfw1@cornell.edu]

Grape berries can become infected by *Botrytis cinerea* at or shortly after flowering. These infections typically remain latent through harvest; however, those few that become active postveraison serve as primary disease foci, from which secondary spread can occur preharvest. Our objective was to investigate environmental and nutritional factors that might influence such latent infection activation and subsequent spread. Potted Chardonnay vines were inoculated with *B. cinerea* at bloom, maintained outdoors but sheltered from rain, and (a) moved to a high-humidity chamber (RH > 92%) for various durations at veraison or preharvest or (b) provided different levels of irrigation postveraison. High humidity did not promote latent infection activation when imposed for up to 9 days at veraison; however, the frequency of active infections increased linearly with increasing humid period duration when it was imposed immediately preharvest. Both activation of latent infections and secondary disease spread were increased in response to increased postveraison irrigation. In a vineyard, individual Chardonnay berries were injected with *B. cinerea* spores at veraison to create disease foci for possible spread, and the vines were then sprayed with urea to increase berry nitrogen without increasing vegetative growth. Disease spread was three times greater in urea-treated versus control vines. We conclude that preharvest rains promote bunch rot epidemics through a variety of mechanisms beyond those related to surface wetness, viz. activation of latent infections via in-

creased atmospheric humidity and soil water availability and increased secondary spread with increased soil water. Secondary spread also is favored by increased berry nitrogen.

Early Diagnosis of Fungicide-Resistant *Botrytis cinerea* in Vineyards by a Nested PCR-RFLP Method

Seiya Saito, Shunji Suzuki,* and Tsutomu Takayanagi
Laboratory of Fruit Genetic Engineering, The Institute of Enology and Viticulture, University of Yamanashi, Kofu, Yamanashi 400-0005, Japan [suzukis@yamanashi.ac.jp]

The increasing occurrence of fungicide-resistant *Botrytis cinerea* isolates is becoming a severe problem for disease control in many plants, including grapes. We collected 81 *B. cinerea* isolates from vineyards over three years. To classify *B. cinerea* isolates into benzimidazole-, phenylcarbamate-, and/or dicarboximide-resistant isolates, fungicide resistance tests were carried out in vitro. DNA sequencing of the genes encoding proteins deactivated by these fungicides demonstrated that a single base mutation was responsible for resistance to these fungicides in fungicide-resistant *B. cinerea* isolates. Based on each mutation, a PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method was developed to identify fungicide-resistant *B. cinerea* isolates. Classification of the isolates by the PCR-RFLP method was consistent with that of in vitro fungicide resistance tests. Then, a nested PCR-RFLP method was developed to detect fungicide-resistant *B. cinerea* isolates at an early growth stage of grapes. This method successfully detected fungicide-resistant isolates of *B. cinerea* in immature grape berries and in young leaves. We propose that early diagnosis of fungicide-resistant *B. cinerea* isolates may contribute to further improving integrated pest management against *B. cinerea* in vineyards.

Clonal Propagation of the Berry Shrivel Disorder and Absence of a Crop-Load Effect

Mark Krasnow, Ken Shackel,* and Mark Matthews
Department of Viticulture and Enology, University of California, Davis, CA 95616 [kashackel@ucdavis.edu]

The berry shrivel (BS) disorder is characterized by poor sugar and pigment accumulation and by shriveling of the fruit during ripening. Fruit from young Cabernet Sauvignon vines propagated from BS wood sources had lower Brix and skin anthocyanin than fruit from vines propagated from control wood sources throughout the season, although the fruit did not exhibit shriveling symptoms at any point. This suggested a potential pathogenic cause of the disorder, but tests for phytoplasmas and leafroll viruses 1-5, 7, 9, 2RG gave negative results. BS fruit from several Cabernet Sauvignon and Sauvignon blanc samples had lower Brix, pH, and berry weight than fruit from neighboring control vines regardless of the site or variety. In Cabernet Sauvignon BS fruit, there was a consistent reduction in anthocyanins compared with control samples. Skin tannin had more inconsistent results, being higher in BS fruit than control fruit from some sites and lower in BS fruit from other sites. A crop-thinning trial was set up in three North Coast vineyards to assess the efficacy

of this technique in reducing or eliminating BS and/or another ripening disorder, bunchstem necrosis: 50% of the crop was removed at two times during the season (set and veraison). At the end of the season, these thinning trials had slight effects on Brix but no significant effect on the incidence of either BS or bunchstem necrosis.

Distribution of Free and Bound Monoterpene Alcohols and Norisoprenoids in Pinot noir Grape Must and Skin

Xiaofen Du, Changrong Ou, and **Michael Qian***
Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331 [michael.qian@oregonstate.edu]

The aroma potential of a red wine derives from aromatic free volatiles and nonvolatile precursors in grape must and skin. Although nonvolatile precursors are odorless, they could be hydrolyzed by enzyme or acid during winemaking or aging to release free aglycones. Monoterpene alcohols and norisoprenoids have the greatest contribution to the varietal aroma of many wines. The objective of this study was to investigate the distribution of free and bound monoterpene alcohols and norisoprenoids in Pinot noir grape must and skin. In grape must, free aroma compounds were extracted with stir bar for 3 hr containing 30% sodium chloride, then thermally desorbed onto a GC-MS for analysis; flavor precursors were isolated with C18 SPE column, and then hydrolyzed by Macer8 FJ enzyme in 0.2 M citric acid buffer at pH 3.1 at 45°C for 20 hr. The freed aglycones were analyzed with SBSE-GC-MS. Grape skin was blended under liquid nitrogen, and then extracted with citric acid buffer. The extracted free aromas and precursors were analyzed with the same procedure as in the must. The results showed that the majority of monoterpene alcohols and norisoprenoids existed in bound forms. In grape must, only small amounts of free forms were found for most terpene alcohols; however, high level of geraniol existed in the free form. In grape skin, almost all aroma compounds existed in a precursor form, except citronellol, nerol, and geraniol. Grape skin had high levels of monoterpene alcohol precursors but low levels of norisoprenoid precursors compared with grape must.

Identification of Key Odorants in Sauvignon blanc with Aroma Reconstitution and Omission Tests

Frank Benkwitz, Paul A. Kilmartin, Cynthia Lund, and Laura Nicolau*
University of Auckland, Department of Chemistry, Auckland, New Zealand [l.nicolau@auckland.ac.nz]

The role of volatile thiols as impact odorants in Sauvignon blanc wines has been shown by several groups. However, after the quantification of 80 Sauvignon blanc wines for their concentration of about 27 volatile compounds which were ranked based on their weighed and normalized odor activity values (OAV), we have shown that, depending on the origin of the wines, some fermentative esters can be ranked as of similar to greater importance to aroma compared with the varietal thiols. Knowing the limitation of using OAV to explain the aroma of complex mixtures, the study was extended to include an aroma extract dilution analysis (AEDA) followed by aroma reconstitution and omission tests. AEDA results showed that flavor dilution (FD) factors vary depending on the solvent used for extraction. However, with few exceptions the same compounds as calculated by OAV were ranked as having a similar high impact in the overall aroma. The role of 20 volatile compounds was studied by aroma reconstitution and omission tests with a deodorized Marlborough Sauvignon blanc wine using a sensory panel specifically trained for assessment of

Sauvignon blanc. Results showed that some compounds with high OAV or FD factors, including 3-mercaptohexanol, 3-mercaptohexyl acetate, and isoamyl acetate do not change the aroma profile of the reconstituted wines as much as compounds with low OAV or FD factors. Fermentative esters were generally found to increase the perception of descriptors associated with volatile thiols, and a masking effect of ethyl hexanoate for banana lolly and apple lolly descriptors was also observed.

Stability of Passion Fruit Type Aromas in New Zealand Sauvignon blanc Wines

Mandy Herbst, Laura Nicolau, and **Paul A. Kilmartin***
University of Auckland, Department of Chemistry, Wine Science Programme, Auckland, New Zealand [p.kilmartin@auckland.ac.nz]

3-Mercaptohexan-1-ol (3MH) and 3-mercaptohexan-1-ol acetate (3MHA) are volatile thiol compounds that contribute to the distinctive passion fruit-type aromas of Sauvignon blanc (SB) wines, but issues remain about the persistence of these aromas in the bottle. The evolution of 3MHA and 3MH, in relation to polyphenol and antioxidant content, has been monitored in 16 Marlborough SB wines bottled in a research winery under both cork and screwcap over two years. These wines were compared to seven commercially bottled Marlborough SB wines, which had initial concentrations of 3MH from 2000 to 11,000 ng/L, and of 3MHA from 200 to 2300 ng/L, the high 3MHA values being a feature of the Marlborough region. 3MHA was the least stable of the volatile thiols, and after 3 months the concentrations in the research wines had decreased by 40–70%, and had largely disappeared after 12 months. These trends were matched by the loss of glutathione and flavan-3-ols, while the hydroxycinnamates were more stable. In the commercial wines, with better control of oxygen entry at bottling, the 3MHA concentrations declined by 20–45% in the 3 months after bottling. By contrast the levels of 3MH were much more stable, with an average loss of 10% after 3 months, and 40% after 12 months in the research wines. In some cases in both research and commercial wines, a sizeable decline in 3MHA levels over the first 3 months was accompanied by a rise in 3MH levels, likely because of conversion by hydrolysis of 3MHA to 3MH. Closure type did not have a major impact on rate of aroma loss.

Impact of Light Exposure on IBMP Accumulation and Degradation during Berry Growth and Ripening

Imelda Ryona, Bruce Pan, Diego Intrigliolo, Alan Lakso, and Gavin L. Sacks*
Cornell University, Food Research Lab, Geneva, NY 14456 [gls9@cornell.edu]

Leaf removal and cluster exposure are associated with reduced levels of 3-isobutyl-2-methoxypyrazine (IBMP) at harvest. This study assessed the effects of light exposure on IBMP accumulation and degradation by comparing shaded and unshaded clusters within the same vine throughout berry development and ripening. Vine-to-vine variability of IBMP and the correlation of IBMP and malic acid were also evaluated. To perform intravine comparisons of IBMP levels, 27 Cabernet Franc vines were shoot-thinned to create regions of high and low light exposure along the canes. Samples were collected at 10 time points during the growing season starting from 5 days postbloom. IBMP extraction was carried out by HS-SPME analysis and quantified by GCxGC-TOF-MS. Significantly higher levels of IBMP were detectable at all preveraison time points in shaded clusters. The unshaded clusters had 21–44% lower IBMP, and these differences did not increase postveraison, indicating that cluster exposure reduces accumulation of IBMP and does not increase IBMP degradation postveraison. Quantifiable levels (2–7 ppt) of IBMP were observed at 5 days postbloom, the earliest observation of IBMP to date. The majority of IBMP accumulation was observed between weeks 3 to 6 postbloom, and IBMP degradation began 2 weeks before veraison. Significant vine-to-vine variability in IBMP content was observed. IBMP and malic acid show similar patterns of accumulation and degradation during the growing season but are differentially affected by cluster exposure pre- and postveraison.

Efficacy of ABA Application to Enhance Wine Color of Cabernet Sauvignon in a Warmer Growing Region

Sanliang Gu,* Kathryn Kaigas, Mark Kaigas, Guoqiang Du, Sean Jacobs, and Robert Wample
Viticulture and Enology Research Center, California State University, Fresno, CA 93740 [sanliang@csufresno.edu]

Abscisic (ABA) applied to clusters was recently reported to significantly increase fruit color on some table and wine grape varieties. Experiments were conducted to evaluate the efficacy of ABA application to enhance wine color of Cabernet Sauvignon in warmer growing regions such as the San Joaquin Valley of California. Clusters of mature Cabernet Sauvignon were sprayed with 600 ppm ABA at 30 and/or 80% berry coloring in 2007. Fruit was sampled on August 14 and September 10 and analyzed for composition and skin color content and then harvested on August 17 and September 11 for winemaking. Wines were analyzed for general chemistry, pigments, phenolics, and tannin after alcoholic or malolactic fermentation and cold stabilization. Wine color and anthocyanin content increased by approximately 50% by single ABA application at 30% berry coloring and doubled when ABA was applied twice at 30 and 80% berry coloring. Skin anthocyanin content increased at similar levels. Wine hue was lower when fruit was treated by ABA, more so by the double applications. ABA application increased small polymeric pigments and phe-

nolics but reduced tannin content. The large polymeric pigments were not affected by ABA application. Wines made from ABA-treated fruit had slightly higher ethanol content while TA and pH were unaffected. For maximum wine color enhancement of Cabernet Sauvignon in a warmer growing region, ABA should be applied directly to the fruit at 600 ppm twice during 30–80% berry coloring.

Effect of Trellis Type on Leaf Gas Exchange and Dynamics of Storage Carbohydrates in Pinot noir Grapevines

Joey Ratliff and **Carmo Vasconcelos***
Horticulture and Food Research Institute of New Zealand, Blenheim 7240, New Zealand [carom@hortresearch.co.nz]

Five trellis and training systems were compared in their effect on wood carbohydrate reserves at four different phenological stages in Pinot noir grapevines. This trial was performed on established vines planted on a low-vigor valley floor site, in the Willamette Valley, Oregon. The treatments were upright vertical, cane pruned (Guyot); upright vertical, spur pruned (bilateral cordon); Scott Henry, cane pruned; lyre, cane pruned; and Geneva double curtain (GDC), cane pruned. At budbreak, bloom, leaf fall, and dormancy, wood samples were taken from the main trunk of four vines for each trellis system. Sugar concentration in the trunk was highest during leaf fall and lowest at bloom on a dry weight basis. Starch concentration in the trunk was highest during dormancy and lowest during leaf fall and budbreak. There were no significant differences in sugar or starch concentrations between the five trellis systems at any sample date. There was no net gain in carbohydrates for any trellis system during the study. The lyre system had the highest concentration of total nonstructural carbohydrates at bloom followed by Guyot. Trunk volume was highest in GDC and lowest in Guyot. Photosynthetic rate was highest in the Guyot system in July of both years, but was similar in all trellis in August and September. Water use efficiency and maximum quantum efficiency of photosynthesis were highest in the bilateral cordon in one of two years. Transpiration rate and chlorophyll content did not differ between trellis systems. Starch concentration at budbreak was closely related to photosynthesis in August and September. No relationship was found between photosynthesis and soluble sugar concentration in the trunk wood.

Identification of Genes Associated with Ester Formation and Degradation in Yeast Strain BY4742

Stuart Robinson and Linda Bisson*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [lfbisson@ucdavis.edu]

The aim of this study was to identify genes associated with ester formation and degradation in the laboratory yeast strain BY4742. The optimal time of ester production using SPME GC/MS occurred at day 3 using a medium with high arginine and ammonium phosphate supplementation. Ninety-seven deletion mutant strains were chosen using the *Saccharomyces* Genome Database based on their functional homology to four genes (*IAH1*, *EHT1*, *ATF1*, *ATF2*) known to impact ester formation and degradation. The 97 deletion mutant strains are grouped into six main categories based on their functional homology: (1) hydrolase, (2) branched chain amino acid degradation, (3) fatty acid metabolism and (4) lipid particle genes, (5) vacuolar H+-ATPase (VMA), and (6) olfactory detection. Ninety-one genes showed differences versus the wild-type strain. Ethyl acetate is produced most commonly in the VMA genes 13/13 with an average increase of 2.6 times over the wild type strain and least commonly in the lipid particle genes 2/26. The VMA family of genes analyzed also shows average increase in isoamyl acetate of 8.6 times that of the wild type. *YJU3,* a gene in the hydrolase functional homology, showed an increase on average of 10 times wild type across all 21 esters measured.

Kinetics of Yeast Protein Release during Aging of Wine on the Yeast Lees

Jeff D. Rowe, James P. Osborne, and Alan T. Bakalinsky*
Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331 [alan.bakalinsky@oregonstate.edu]

Yeast mannoproteins released during the process of aging wine on the yeast lees have been reported to enhance mouthfeel (texture) through interaction with grape tannins and to contribute to wine quality. In order to better understand these contributions, a model system was developed to follow the kinetics of yeast protein release over the course of 9 months after completion of fermentation. Model musts were fermented in duplicate by a number of commercial yeast strains, including BM45 and RC212, and were stored on the lees with stirring under cellar conditions, postfermentation. Wine samples were taken during and after fermentation, and total protein and total mannoproteins were measured following removal of suspended solids. In addition, individual proteins were identified by HPLC-MS of their tryptic fragments. The total number of identified proteins in all samples increased following fermentation, peaking at about 60 different proteins after one month on the lees, and decreasing after 6 months of aging to about 20. Over 50% of the identified proteins were shared among all yeast samples. One GPI-anchored mannoprotein, Pst1 (YDR055w), found in all samples after the first sampling, was recently reported to reduce haziness in white wines, presumably because of its ability to interfere with aggregation of denatured wine proteins. Sampling will reveal which of these proteins and which mannoproteins among them will continue to remain soluble with the potential to interact beneficially with other wine constituents.

Effect of Dissolved Oxygen Concentration on *Brettanomyces* Growth and 4-Ethylphenol Production

Laura M. Weissberg, C.M. Lucy Joseph, and Linda F. Bisson*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [lfbisson@ucdavis.edu]

Oxygen has been shown to stimulate both growth and acetic acid production of *Brettanomyces* yeasts. This study further examined the role of oxygen with respect to *Brettanomyces* spoilage capacity and sought to determine the effect of dissolved oxygen concentration on growth and 4-ethylphenol (4-EP) production. Secondly, the experiment aimed to determine if the production of 4-EP, from the hydroxycinnamate *p*-coumaric acid, benefits the cells physiologically. The experimental design was a 3 x 3 x 2 factorial composed of three strains of *B. bruxellensis* (UCD 2077, 2082, 2091), three dissolved oxygen conditions (0%, 25%, and 50% air saturation), and 0 or 20 ppm of *p*-coumaric acid. Batch fermentations were carried out for 10 days in bioreactors, containing 4.25 L of minimal media with continual control of the desired air saturation level by the use of in-line dissolved oxygen probes. Daily samples were analyzed for optical density, cell numbers, and 4-EP concentration. Preliminary results illustrated that concentrations of 4-EP by the end of fermentation were affected by strain and by air saturation level. Furthermore, the highest amounts of 4-EP produced per cell were found in the 0% air saturation treatment. In contrast, preliminary data indicated that growth was not significantly affected by 4-EP production. The information revealed by this study aids in understanding the physiological basis of volatile phenol production by *Brettanomyces* sp. and its relation to the redox potential of the environment of the cells.

Role of Wine Yeast Strains in the Production of Positive Aroma Attributes during Winemaking

Ana M. Molina, Victor Guadalupe, and **Eduardo Agosin***
Departamento de Ingeniería Química y Bioprocesos, Facultad de Ingeniería, Pontificia Universidad Católica de Chile, Santiago, Chile [agosin@ing.puc.cl]

The choice of the yeast strain to conduct wine fermentation is critical for the final composition of the resulting wine. To date, there is no definitive information about the magnitude and mechanisms mediating the effect that different commercial yeast strains have on the aromas produced during winemaking. In this work, the levels of volatile compounds synthesized by different commercial strains of *Saccharomyces cerevisiae* (EC1118, VIN13, QA23, X5, RX60, UVAFERM) were quantified by GC/MS after fermentation of chemically defined must in 2-L bioreactors under controlled conditions at 15°C or 28°C. Although most of the strains synthesized similar volatile compounds, significant differences in the concentration of some particular compounds associated to positive aromatic attributes were detected. The sensory description and perception intensity of the volatile compounds were also determined using quantitative gas chromatography-olfactometry. Characteristic sensory features that could facilitate the selection of the appropriate strain to pursue a particular wine style were found for some strains. The transcriptomes (using serial analysis of gene expression) of *S. cerevisiae* EC1118 and VIN 13 strains under the same

culture conditions were compared in an attempt to search for new, noncharacterized, genes related with fermentation performance and the observed differential aroma production. Transcripts corresponding to hypothetical protein, intergenic expressed regions, and not previously reported expressed sequences were found. Their putative role in aroma synthesis and fermentation performance under wine environment will be discussed.

Variation in the Skin Tannin Content, Composition, and Polymer Length Distribution of 36 Different Grape Varieties

Tara J. Seddon, Marica Mazza, Simone Rochfort, Mary Millikan, and **Mark O. Downey***
Department of Primary Industries, Mildura, VIC 3502, Australia [mark.downey@dpi.vic.gov.au]

Grape tannin content and composition studies are limited to a few major varieties, which may have created a misleading generalized impression of grape tannins. We examined tannins in skins of 36 different cultivars by acid-catalyzed cleavage with quantification by HPLC (phloroglucinolysis) and gel permeation chromatography adapted for HPLC (GPC). Tannin levels ranged from 2.30 (Carolina Black Rose) to 15.91 mg/g fwt skin (Sagrantino). Of terminal subunits, catechin ranged from 26.5% (Pinot Gris) to 86.8% (Ribier), epicatechin from 5.6% (Pinot Gris) to 31.9% (Malbec), and epicatechin-gallate from 5.8% (Sagrantino) to 67.9% (Pinot Gris). Consistent with published work, epigallocatechin was not observed as a terminal subunit. Contrary to previous work, in two samples (Pinot Gris, Shiraz) epicatechin-gallate was the most prevalent terminal subunit. Of extension subunits, catechin ranged from 11.5% (Beauty Seedless) to 31.8% (Brown Muscat), epicatechin from 38.8% (Flame Seedless) to 67.4% (Pinot noir), epicatechin-gallate from 12.2% (Pinot noir) to 48.8% (Beauty Seedless), and epigallocatechin from 0.4% (Cardinal) to 1.8% (Cabernet Sauvignon, Cienna). High galloylation and low epigallocatechin content was generally observed, which may be a regional effect. It is uncertain what impact changes in subunit composition may have on wine sensory character. Average polymer length (phloroglucinolysis) ranged from 9.5 (Pinot Gris) to 45.7 subunits (Flame Seedless). In the GPC fraction containing the largest polymers, lengths ranged from 38.1 (Barbera) to 112.4 subunits (Flame Seedless). Polymer lengths were in many cases greater than previous reports. Frequently higher levels of total tannin and longer polymer lengths were observed in table grapes.

Survey of Changes in Free Anthocyanin Accumulation at Different Vegetative Vigor Levels in *Vitis vinifera* L. Cabernet Sauvignon

Gerard Logan,* Petra King, Helen Searle, Malcolm Reeves, and Daniel McClellan
Eastern Institute of Technology, Taradale 04142, New Zealand [GLogan@eit.ac.nz)]

Due to inherent within vineyard variability of microclimate, differences exist in the vegetative vigor of vines. Fruit composition and color can be affected by unbalanced vines. Free anthocyanins are responsible for young red wine color and are often the first attribute to be judged by a consumer. In unbalanced vines, the production of anthocyanins can be altered and the perceived wine quality affected. The objective of this work was to determine if vigor differences had an effect on anthocyanin accumulation and final concentration in the fruit of *V. vinifera* L. Cabernet Sauvignon. The experimental design included a randomized block of *V. vinifera* L. Cabernet Sauvignon on the Gimblett Gravels in Hawkes Bay, with vines differentiated into three vigor levels using the NDVI measurement technique, trunk circumferences, and pruning weights. The entire block was treated uniformly with regard to viticulture. Cluster samples were taken three times during the period between veraison and

harvest and processed for HPLC analyses. Data suggested that although red pigments decreased with increasing vine vigor, anthocyanin concentration was higher in medium vigor vines. Additionally, individual anthocyanins behaved differently: delphinidin-3-*O*-glucoside decreased with increasing vigor while malvidin-3-*O*-glucoside increased. This is one parameter which suggests that differential management and fruit harvest may prove useful in vineyards with variable vine vigor.

Tannin Accumulation and Composition during Grape Berry Development

Rachel Hanlin and Mark Downey*
Primary Industries Research Victoria, University of Adelaide, Mildura, VIC 3502, Australia [mark.downey@dpi.vic.gov.au]

Condensed tannins derived from the grape berry play a significant role in wine astringency, bitterness, color stability, and aging potential. Understanding the regional, varietal, and seasonal variability in winegrape tannin composition and accumulation throughout berry development is essential for the development of vineyard strategies to manage and manipulate winegrape tannin to meet winery specifications. The current understanding of tannin accumulation and composition in grape berries is largely based on a single variety and geographical region with most studies investigating seed tannin. To investigate the variation in tannin accumulation and composition, Shiraz and Cabernet Sauvignon grape skin from two growing seasons in Sunraysia, southeast Australia were measured by acid-catalyzed cleavage in the presence of phloroglucinol. HPLC was used to determine the subunit composition, total tannin content, and average polymer length throughout berry development. The pattern of tannin accumulation and composition were similar for both varieties and seasons with tannin content highest at fruit set before declining to veraison. The pattern of accumulation and composition seen in grape skin from this study was different than that previously seen in Shiraz from the cooler growing region of McLaren Vale in South Australia, where tannin content was highest at veraison. Results indicate that the differences seen between the pattern of tannin accumulation and composition in Sunraysia and McLaren Vale may be dependent on regional differences between sites and has a more significant impact than varietal or seasonal variability. Further research is required to determine the full extent of variation between regions and to determine the environmental factors that can be used to influence the pattern of tannin accumulation.

Assessing the Impact of Temperature on the Development and Composition of Grape Berries (*Vitis vinifera* L. cv. Merlot)

Seth D. Cohen, Julie M. Tarara, and James A. Kennedy*
Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331 [james.kennedy@oregonstate.edu]

The importance of temperature on the development and composition of winegrapes is evident in the use of a growing degree day model to predict the success of a cultivar within a growing region. However, there are many aspects of temperature whose affect on plant growth are poorly understood (e.g., diurnal temperature range, daytime versus night-time temperature). This study assessed the impact of fruit tem-

perature on the phenological development and phenolic metabolism of grape berries (*Vitis vinifera* L. cv. Merlot) grown under field conditions. Individual cluster temperatures were manipulated while controlling for exposure to sunlight. In-situ berry temperatures were collected for detailed analysis of temperature profiles. Daytime and nighttime temperature controls were applied separately and in combination to dampen the diurnal temperature fluctuation. Samples were collected at veraison and harvest and assessed for physiological and compositional differences. Chemical analysis included quantification of anthocyanins, flavonols, flavan-3-ol monomers, and proanthocyanidins. Metabolites showed variable responses to treatments and timing of treatments. Samples collected at veraison showed hastening of ripening from daytime cooling and dampening the diurnal temperature range. Treatments applied from veraison to harvest, 2006, resulted in minimal differences in berry physiology or composition. Treatments were applied for the entire 2007 growing season to determine if differences observed early in berry development translated to differences at commercial maturity. Results from this study are intended to assess the ramifications of manipulating fruit temperature with regard to berry composition and time required for commercial ripening.

Field Temperature and Anthocyanins in Merlot Grape Berries

Julie M. Tarara,* Jungmin Lee, Sara E. Spayd, and Carolyn F. Seagael
U.S. Department of Agriculture–ARS, Prosser, WA 99350 [jtarara@wsu.edu]

On field-grown vines, temperatures of Merlot grape clusters were monitored and controlled from preveraison until harvest to produce a dynamic range of berry temperatures in both sun-exposed and shaded fruit. Ten combinations of temperature and solar radiation exposure were applied, and resulting phenolic profiles and total concentrations of skin anthocyanin (TSA) in the fruit were qualified at commercial maturity. Concentrations of flavonol-glycosides increased with exposure to solar radiation. Low light and high berry temperatures in concert decreased TSA rather than low incident solar radiation alone, which appeared not to compromise total anthocyanin accumulation. At higher berry temperatures, malvidin-based anthocyanins were at higher concentrations and comprised a larger proportion of TSA, regardless of cluster exposure to solar radiation. Acylated anthocyanins were a larger proportion of TSA than nonacylated anthocyanins under high temperature extremes in sunlit and shaded fruit and under shade alone. When berry temperature was held equal to that of shaded fruit, exposure to solar radiation decreased the proportion of TSA in acylated derivatives of all five base anthocyanins and increased the proportion of TSA comprised by dihydroxylated anthocyanins. There appears to be a complex combined effect of solar radiation and berry temperature on anthocyanin composition in the field that is synergistic at moderate berry temperatures and potentially antagonistic at high temperature extremes. Exposure of berries to high temperature extremes for relatively short periods during ripening appears to alter the partitioning of anthocyanins between acylated and nonacylated forms and between dihydroxylated and trihydroxylated branches of the anthocyanin biosynthesis pathway.

Interaction of Polyphenols, Oxygen, and Sulfite in Wine

John C. Danilewicz*
Ash, Canterbury, Kent CT3 2AF, United Kingdom [jdanilewicz@btconnect.com]

The reaction of sulfite and oxygen at wine pH is a metal-catalyzed radical-chain process. Radical scavengers, such as catechols, prevent radical-chain propagation and so inhibit the reaction, which consequently should not occur in wine. Sulfite cannot therefore exert its antioxidant action in wine by reacting with oxygen but does so by removing any hydrogen peroxide that is produced when polyphenols are oxidized. In support of this conclusion is the observation that, in a model wine system, the rate of sulfite reaction is dependent on the concentration of a catechol, which determines the rate of production of hydrogen peroxide on oxidation. If not intercepted, hydrogen peroxide rapidly oxidizes ethanol to acetaldehyde by way of the Fenton reaction. Catechols also cannot react directly with oxygen and do so through the intermediate catalytic intervention of iron and copper. In the presence of 4-methycatechol in a model wine that contains both metals, the sulfite/oxygen molar reaction ratio is 1:2. Sulfite must therefore also be reacting with the quinone, ~40% of which undergoes nucleophilic addition to produce the sulfonic acid and most of the remainder is reduced back to the catechol. When the quinone is intercepted by benzenesulfinic acid, the sulfite/oxygen molar reaction ratio is reduced to 1:1. Sulfite and benzenesulfinic acid accelerate the oxidation of catechols, and so it is proposed that nucleophiles, by intercepting quinones, drive oxidation forward. These mechanistic considerations are relevant to understanding winemaking processes such as microoxygenation, which may be conducted in the presence or absence of sulfite.

Sensory and Chemical Changes Induced by Oxygenation during and after Fermentation in Pinot noir and Cabernet Sauvignon Wines

Dominik Durner,* Fabian Weber, Michael Rentzsch, Peter Winterhalter, and Ulrich Fischer
DLR Rheinpfalz, Department of Oenology and Viticulture, D–67435 Neustadt/Weinstrasse, Germany [dominik.durner@dlr.rlp.de]

Insufficient exposure of red wines to oxygen in large-scale stainless-steel containers is considered disadvantageous for their sensory qualities. Since the aging of red wine is strongly associated with the oxygen-induced formation of polymeric compounds, the controlled continuous supply of oxygen is used as a technique to accelerate the ripening of red wines. Our objective was to compare the impact of a short-term microoxygenation treatment with high oxygen dosage during alcoholic fermentation and long-term treatment with low oxygen dosage after malolactic fermentation. Cabernet Sauvignon and Pinot noir wines from vintages 2006 and 2007 were chosen due to their substantial variation in anthocyanin to catechin ratio. Descriptive analysis of Pinot noir wines revealed smaller reductions in color and more distinctive enhancement of fruity attributes due to an early oxygen treatment. The overall decrease in bitterness and tannin perception was similar in both treatment periods. Applying the modified Adams assay for phenolics in wine, Pinot noir showed a faster development of large polymeric pigments than Cabernet Sauvignon, which may be due to the over-proportional presence of catechin in this grape variety. Analysis by

HPLC-DAD proved that major monomeric flavonoids of both grape varieties were more affected by late oxygen treatment. Untargeted mass spectrometry techniques verified that added oxygen after malolactic fermentation resulted in higher levels of ethylidene-bridged polyphenols. These compounds were also found at low levels when oxygen was solely applied during alcoholic fermentation; however, the same concentrations were detected in untreated control wines.

Influence of Harvest Date on the Sensory Profiles of Icewines from the Niagara Peninsula

Amy J. Bowen,* Andrew G. Reynolds, and Isabelle Lesschaeve
Cool Climate Oenology and Viticulture Institute, Brock University, St. Catharines, ON L2S 3A1, Canada [amyjbowen@hotmail.com]

Icewine is a sweet dessert wine made from pressing grapes naturally frozen on the vine. The Vintners Quality Alliance in Ontario regulates icewine production, including specifying harvesting after 15 November. We hypothesized that grapes destined for icewine go through a series of freeze and thaw cycles that change their chemical and sensory profiles due to climatic events. The objective of this study was to determine the influence of harvest date on icewine sensory profiles. Riesling and Vidal icewines were made from grapes picked between December 2004 and February 2005: harvest 1, 19 Dec; harvest 2, 29 Dec; harvest 3, 18 Jan; harvest 4, 11 Feb (Vidal only). Triangle tests showed significant differences among harvest dates for both cultivars ($p < 0.05$). Wines were then evaluated by descriptive analysis using 14 trained assessors. Ten and 11 aroma and flavor attributes were significantly different ($p < 0.05$) in Vidal and Riesling icewines, respectively. For Vidal, later harvest dates had significantly higher intensity scores for aroma and flavor descriptors than harvest 1. For Riesling, harvest 1 wines had higher intensity ratings for fresh fruit descriptors, whereas harvest 3 wines were higher for dried fruit and nutty descriptors. Principal component analysis (PCA) on Vidal found all significant attributes associated with harvest 3 and 4. In Riesling, PCA showed fresh fruit attributes associated with harvest 1 and 2, whereas dried fruit and nutty descriptors associated with harvest 3. Harvest date was identified as a discriminating dimension using canonical variant analysis in Riesling; however, this was not found for Vidal.

Modulation of Shiraz Volatiles, Color, and Aroma Aging Potential through Di-Ammonium Phosphate Addition

Maurizio Ugliano,* Tracey Siebert, Dimitra Capone, Meagan Mercurio, and Paul A. Henschke
Australian Wine Research Institute, Glen Osmond SA 5064, Australia [maurizio.ugliano@awri.com.au]

Supplementation of low nitrogen grape musts with yeast assimilable nitrogen (YAN), particularly in the form of di-ammonium phosphate (DAP), is frequently carried out to optimize yeast fermentation performance and assist in the control of hydrogen sulfide (H_2S) formation. However, the effect of DAP supplementation of red grape musts that contain grape solids on wine volatile composition has not been investigated. In this study, a Shiraz must with low YAN was supplemented with two concentrations of DAP and then fermented with maceration on grape skins. The concentration of volatile and nonvolatile compounds was determined in the wines after bottling. DAP addition had a strong impact on the volatile composition of Shiraz wines. Variations in the concentration of different classes of compounds, such as medium-chain fatty acids and their esters, acetates, higher alcohols, isoacids and their ethyl esters, were observed when the initial YAN content of the must was increased. Conversely, DAP had no effect on the final concentration of

H_2S and dimethyl sulfide at bottling. An effect on polyphenolic constituents and color parameters of the final wines was also observed. Aging of the wines caused significant changes in the concentration of the various classes of esters analyzed. Dimethyl sulfide was found to increase with aging, its final concentration depending on the initial concentration of DAP added to the must. This work suggests that DAP supplementation of a low YAN must fermented by maceration on skins can significantly impact on the sensory properties of red wine.

Sensory and Chemical Changes Induced by Secondary Fermentation of Riesling and Chardonnay Sparkling Wines

Sebastian Ganß, Hans-Georg Schmarr, Jens Witte, Peter Winterhalter, and **Ulrich Fischer***
DLR-Rheinpfalz, Department of Oenology and Viticulture, D–67435 Neustadt/Weinstrasse, Germany [ulrich.fischer@dlr.rlp.de]

During secondary fermentation, sensory properties of base wines are usually improved. Occasionally however, formation of off-flavors during this process may spoil sparkling wines and lead to financial losses. Currently, base wine selection is solely based on expert experience rather than on scientific knowledge. Sensory analysis of base wines and their subsequent sparkling wines suggested that secondary fermentation in general decreases green odor impressions and in return enhances those reminiscent of ripe fruit. Application of gas chromatography-olfaction (GCO) revealed enhanced sensory activities for a range of fruity esters and fusel alcohols after secondary fermentation. At the same time only a few aroma compounds were diminished. The role of aroma precursors in Riesling and Chardonnay were investigated by adding precursor fractions to both base wines prior fermentation, which were obtained from the respective varieties using an XAD-2 resin. Due to precursor supplementation, a significant enhancement of color, peach, elderberry blossom, green banana, and green bean intensities were observed in the finished Riesling sparkling wine. For Chardonnay color, honey melon and grape juice perception was increased. The molecular base for these sensory changes could be detected by a build-up of aroma compounds such as 2-phenylethanol, (Z)-hex-3-en-1-ol, linalool and ß-damascenone. Although base wines for sparkling wine production lack the degree of ripeness of normal still wines, it could be demonstrated that the release of aroma compounds from precursors in the respective base wines explains partially the flavor changes observed during secondary fermentation and suggests placing more emphasis on their role when composing a cuvee for sparkling wine production.

Chemical and Sensory Effects of Wineglass Shape

Gregory Hirson, Hildegarde Heymann, and Susan Ebeler*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [seebeler@ucdavis.edu]

The effects of wineglass shape and equilibration time before sampling were investigated using headspace solid-phase microextraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS). The headspaces of covered wineglasses were

extracted for 1 min using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber after equilibration times of 0 min, 5 min, and 10 min. Changes in headspace composition (on a percent of total volatiles basis) were attributable to glass shape, equilibration time, and a glass*equilibration time interaction. Sensory descriptive analysis (DA) was used to evaluate sensory differences between wineglasses, equilibration times, and wineglass*equilibration time interactions. Principal component analysis (PCA) and partial least squares regression (PLS) were used to relate changes in headspace composition to changes in sensory descriptive observations.

Human Differences in Detection of *Brettanomyces* Odor Impact Compounds

C.M. Lucy Joseph, Susan E. Ebeler, Hildegarde Heymann, and Linda F. Bisson*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [lfbisson@ucdavis.edu]

The olfactory receptor (OR) genes comprise the largest gene family in the human genome. There are over 300 verified OR genes that are divided into 17 families and 127 subfamilies based upon gene sequence and putative protein structural similarities. Different alleles of these genes also exist, indicating that the human perception of aroma can be quite variable. During the analysis of the impact of specific precursor compounds on odor impact compounds produced by the spoilage yeast *Brettanomyces*, it became apparent that there were physiological differences in the ability to detect certain specific compounds produced by this organism. Odorants were chemically identified from cultures of different strains of *Brettanomyces* with specific supplements. Only those compounds that were detected consistently by the majority of the nine panelists were subsequently identified. The ability of nine panelists to detect those specific compounds in a model solution was investigated using GC-olfactory technology. A variety of responses were seen. The number of compounds detected ranged from 100% to 77%, depending upon the compound/panelist. Some compounds were detected by all panelists, one compound by only five. Panelists also provided descriptors of the aromas that were being detected. In some cases there was excellent reproducibility across replicate runs in the aromas that were detected. In other cases panelists were able to reproducibly detect the compound, but the descriptor used varied widely. Some descriptors changed for a given panelist depending upon the day they sniffed the samples. In still other cases a panelist detected compounds on one day but not on another.

From Fumigant to Tracer: How Sulfur Inputs Provide Insight into Vineyard Water Losses

Eve-Lyn S. Hinckley,* Carol Kendall, and Keith Loague
Department of Geological and Environmental Sciences, Stanford University, Stanford, CA 94305 [ehinckley@stanford.edu]

The common use of native sulfur (S) in vineyards as a fumigant or soil additive provides a novel way to isotopically differentiate among S pools, allowing the estimation of water losses and S budgets. As part of a 2.5-year study, the research objectives were to (1) characterize the near-surface hydrological flow paths during irrigation and storm events in a Napa Valley vineyard and (2) determine how those flow paths affect the fate and transport of S across seasons. Integration of hydrological theory with measurements of sulfate concentration (expressed as $[SO_4^{2-}]$) and sulfate-S isotopic ratios (expressed as $\delta^{34}S$) in inputs, soil water, and leachate provided a means of determining hydrological flow paths. Low (SO_4^{2-}) and $\delta^{34}S$ in leachate during 4-hr irrigation events reflect minimal engagement of the soil matrix, indicating that preferential flow was the dominant path for water in the near surface. In contrast, high (SO_4^{2-}) and $\delta^{34}S$ values during 8-hr irrigation and storm events reflect near complete engagement of the soil matrix, indicating that lateral flow was the dominant pathway. These results suggest that (1) preferential flow is an important loss pathway to consider in managing water and nutrients in vineyards and that (2) over the long-term, continued S use may have potentially negative consequences for soil and water quality both on and off site.

Evapotranspiration-Based Irrigation Scheduling for Syrah in California: Assessing Vine Water Status by Petiole Electrical Potential

David Sorokowsky, Andrew G. Reynolds,* and William Gensler
Cool Climate Oenology & Viticulture Institute, Brock University, St. Catharines, ON L2S 3A4 Canada [areynold@brocku.ca]

Six irrigation treatment combinations of three evapotranspiration (ET) values (100%, 50%, 25%) and two crop coefficients [Kc; 0.6 and 0.2–0.8 (variable)] were imposed on Syrah/110R vines in the Dunnigan Hills in a randomized block containing four replicates and 12 equally spaced vines per three-row treatment replicate. Treatments began at fruit set and continued until one week before harvest. The variable treatment was not carried out in 2006. Midday leaf water potentials (ψ) were lowest in the 25ET/variable vines (frequently <-15 bars). Petiole conductance (measured by a Phytogram platinum probe in the petiole) followed the ψ pattern, suggesting this technology's potential for monitoring vine water status remotely. Irrigation had no effects on most yield components. Berry weight was highest in the 100ET/variable and lowest in the 25ET treatments. Soluble solids were highest in 50ET and 25ET treatments in 2004 and 2006; in 2005, 25ET treatments were lowest. Titratable acidity (TA) was lowest in 25ET and 50ET/variable treatments in 2004 and 2006; 50/0.6 berries were highest. Highest pH in 2004 and 2006 occurred in 25ET/0.6 and was lowest in 100ET/variable and 50ET/0.6 berries. Anthocyanins were lowest in 50/variable and 25/0.6 in 2005 and highest 100/0.6 in 2006. Phenols were highest in 25ET treatments in 2004 and lowest in 25/0.6 in 2006; the 50/0.6 treatment

was highest in 2005. Sensorially, 25ET treatments diminished red fruit aroma/flavor without impacting intensity of other fruit components and had least vegetal and most lavender and chocolate aromas; retronasally, they had most dark fruit, anise, alcohol/heat, viscosity, and length.

Vine Pigment Reaction to Water Stress with the Incorporation of Conventional and Hyperspectral Measurement Techniques

Albert Strever,* E. Potgieter, and P.R. Young
Stellenbosch University, JH Neethling Building, Stellenbosch, Western Cape 7600, South Africa [aestr@sun.ac.za]

Water stress measurements in vineyards are mostly based on localized soil water content measurements or occasionally on vine-based measurements such as leaf water potential or stomatal conductance. Nondestructive measurements such as leaf temperature and pigment fluorescence already show potential to complement conventional measurements in determining plant water stress, but routine application in viticulture remains difficult because of the complexity and scale of the measurements needed. The objectives of this study were to use hyperspectral field spectroscopy to find spectral bands of interest when measuring vine water status in the field, which could benefit future developments with regard to aerial or satellite remote sensing or handheld instruments for water-stress monitoring. An irrigation trial layout had been designed at a *Vitis vinifera* cv. Merlot vineyard in Stellenbosch, where field spectroscopy, leaf pigment extraction, and HPLC analysis were performed along with conventional plant and soil water status measurements. The interactions found between chlorophylls, carotenoids, vine vigor, and water stress levels are discussed together with general results from plant measurements and microvinification.

Relationship between Grapevine Water Use and Dry Biomass Production and Nitrogen and Potassium Uptake

Larry E. Williams*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [williams@uckac.edu]

It has been demonstrated in many plant species that dry biomass production is a linear function of water use; however, such a relationship has not been shown for grapevines (*Vitis vinifera* L.). Leaves, stems, and clusters on Thompson Seedless grapevines were harvested various times during the growing season over a 3-yr period, dried, and weighed. Water use of grapevines was calculated at each harvest, summing the amount of water applied and that lost from the soil profile. Seasonal water use at fruit maturity averaged 715 mm across years. Dry biomass increased linearly with water use. The relationships between N and K uptake in the shoots and grapevine water use were curvilinear. During the second year of the study, entire root systems of the grapevines were excavated from the soil and the total root and trunk biomass and N and K in those organs quantified. The decrease in root total N between the first of April and the middle of July would account for ~80% of the N found in the organs of the shoots not associated with being taken up by the vine via the transpiration stream. By fruit maturity, total N in the root system was similar to

that at the beginning of the season. Little K was lost from either the root system or trunk during the season. The results indicate an active uptake of K by the vines from the soil and that N from the roots could be used to supplement N from the soil to support growth of the shoots.

Influence of Irrigation Strategies on Photosynthetic Rates of Syrah Grapevines

Dan Rodrigues* and Keith Patterson
Department of Horticulture and Crop Science, California Polytechnic State University, San Luis Obispo, CA 93407 [dmrodrig@calpoly.edu]

The focus of this study was to determine the influence of differing degrees of deficit irrigation (RDI) on gas exchange of Syrah grapevines grown in the Paso Robles AVA. The study used a gas analyzer (LiCor 6200) to measure the overall rate of photosynthesis during two successive growing seasons (2004, 2005). Measurements were taken from the bloom period through harvest and were compared among four different irrigation levels (75, 60, 45, and 30% ET). The 60% level was considered the control, as it is the grower standard in this region. Along with the overall photosynthetic rates, leaf temperature, stomatal conductance, light levels, and relative humidity were also recorded. The results of this 2-yr study showed that minor differences in the mean photosynthetic rates were found between the four different irrigation levels. However, no statistical significances were found, possibly because of the weak stomatal control of transpiration reported for this variety. A significant difference was observed in the measured gas exchange rates between sun exposed and shade leaves within each treatment.

Role of Embolism in the Drought Response of Grapevine

William Drayton and Mark Matthews*
Department of Viticulture and Enology, University of California, Davis, CA 95616
[mamatthews@ucdavis.edu]

Manipulating the water status of cultivated grapevines is an important tool to control vegetative and reproductive growth. To maximize the benefits from deficit irrigation, it is important to understand the physiology of water transport under stress. Water is transported through xylem conduits under tension and is therefore susceptible to cavitation. As water deficits develop, tension, and hence, cavitation, increases. Cavitation leads to the formation of gas bubbles (or embolisms) that block xylem conduits, decrease the hydraulic conductivity (K_h), and reduce water transport to the leaves and fruit. It has been hypothesized that grapevines are particularly vulnerable to water-stress-induced embolism because of their large xylem conduit diameters. However, grapevines are generally considered drought-tolerant crops, and midday leaf water potential values rarely reach below -1.7MPa. Two techniques were used to test the vulnerability of stems of *Vitis vinifera* cv. Chardonnay to embolism and reduced K_h; the common air injection technique and a bench-top drying technique. K_h was then assessed by measuring the flow of water through the stem at constant pressure. Stems were highly resistant to embolism induced by either technique, with 50% loss in K_h not occurring until -2.5MPa and 100% loss at -3.5MPa. Similar results were obtained with stems from field- and greenhouse-grown vines. Results suggest that grapevine is resistant to stem embolism considerably outside its typical physiological range of xylem tension. We discuss the results in the context of other published work on K_h in winegrape and the context of drought tolerance mechanisms in grapevines.

Influence of Regulated Deficit Irrigation and Crop-Level Management on Fruit Composition and Wine Sensory Characteristics

Sara E. Spayd,* Hildegarde Heymann, Robert Wample, and Robert Evans
Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695
[sara_spayd@ncsu.edu]

Vines of *Vitis vinifera* L. cvs., Cabernet Sauvignon, Riesling, and Chenin blanc were studied for three years to determine the interactive effects of varying levels of water deficit and crop level on fruit composition. Sensory characteristics of Cabernet Sauvignon wines were assessed using descriptive analysis. Three irrigation levels and crop levels were imposed as described in a related abstract. Fruit maturation was advanced as degree of water deficit increased and crop level decreased. However, the magnitude of delay was dependent on cultivar. Canonical variate analyses (CVA) were used as a mean separation technique for the multivariate analysis of variance. The interaction of irrigation and cropping level CVA were significant at $p < 0.05$ according to Roy's Greatest Root, and these were used to interpret the results for each year. Cabernet Sauvignon wines from vines that received the most supplemental irrigation water (HI) and carried the largest crop level (HC) differed in sensory characteristic from all other combinations of irrigation and crop-level treatment. The HI-HC wines were associated with the descriptive terms cooked cabbage, green olive, and stemmy.

Influence of Regulated Deficit Irrigation and Crop-Level Management on Soil and Vine Water Status and Vegetative and Reproductive Growth

Sara E. Spayd,* Lynn Mills, Robert Wample, and Robert Evans
Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695 [sara_spayd@ncsu.edu]

Vines of *Vitis vinifera* L. cvs., Cabernet Sauvignon, Riesling, and Chenin blanc were studied for three years to determine the interactive effects of varying levels of water deficit and crop level on vegetative and reproductive growth. Base soil moisture levels within the top 1 m of soil (SM_{lm}) for the high, medium, and low irrigation treatments were 6.1, 4.6, and 3.8 cm, respectively. The irrigation treatments were: high irrigation (HI) = [(6.1cm – SM_{lm}) + (K_c x ET_g)], medium irrigation (MI) = [(4.6cm - SM_{lm}) + (0.75 x (K_c x ET_g))], and low irrigation (LI) = [(3.8cm – SM_{lm}) + (0.50 x (K_c x ET_g))]. Crop coefficients (K_c) developed for *V. vinifera* were used in conjunction with evapotranspiration for a grass reference crop (ET_g). Cluster thinning at pea size/bunch closure was used to reduce crop level. During the first year of the study, either no clusters, one-third clusters, or two-thirds clusters were removed. In the latter two years, either no clusters were removed or either about 35 or 70 clusters/vine were retained. Increasing degree of water deficit reduced leaf size, leaf area/shoot, leaf area/vine, yield, berry weight, and leaf area/fruit weight, while crop load increased. Increasing crop level increased yield and crop load. Cultivars did not respond to irrigation or crop level in a similar manner for a number of measurements.

Impact of Vine Water Status on the Sensory Profile of Riesling Wines

James J. Willwerth,* Andrew G. Reynolds, and Isabelle Lesschaeve
Cool Climate Oenology and Viticulture Institute, Brock University, St. Catharines, ON L2S 3A1 Canada [j_willwerth@hotmail.com]

We sought to elucidate potential determinants of terroir that impact wine varietal character by choosing vine water status as a major factor of the terroir effect. One hypothesis was that consistent water status zones could be identified within vineyard sites and that differences in wine sensory attributes could be related to vine water status. To test this hypothesis, 10 Riesling vineyards located in the Niagara Peninsula were delineated using global positioning systems (GPS) and 75–80 sentinel vines were geo-referenced within a sampling grid for data collection. Vine water status measurements (midday leaf ψ) were collected biweekly during the 2005–2007 growing seasons from a subset of these sentinel vines. Geospatial maps were used to segregate vines from "low" and "high" water status regions within each vineyard block, and replicate wines were made from each region. A sorting task was performed on wines from the 2005 vintage. Using sorting tasks and multidimensional scaling, wines of similar water status were shown to have similar sensory properties. Descriptive analysis using a trained panel further indicated that water status had an effect on wine sensory profiles. In several vineyards, similar attributes were significantly different for wines of different water status ($p < 0.05$). Wines made from vines of high water status had more intense aroma scores for baking spice and honey aromas and apple/pear and honey flavors, whereas those of low water status had more intense flavor scores for citrus, peach, tropical fruit, and sour taste.

Impact of Vine Vigor on Berry Parameters and Free Anthocyanin Extraction in Hawkes Bay *Vitis vinifera* L. Syrah

Gerard Logan,* Petra King, Helen Searle, Malcolm Reeves, and Daniel McClellan
Eastern Institute of Technology, Taradale 04142, New Zealand [GLogan@eit.ac.nz]

As color is normally the first impression a consumer has of wine quality, color chemistry is a large focus in modern red grape and wine research. Free anthocyanins make up a large proportion of the color of a young red wine. The Hawkes Bay region of New Zealand is increasingly renowned for producing high-quality Syrah, and thus a greater understanding of viticultural impacts is desired for constant improvement. The aim of this research was to determine the impact of vine vigor on a relationship between berry weight, skin thickness, and free anthocyanin in the fruit, and what effect this had on final wine color. The trial was set up in a randomized complete block design and vegetative vigor level was determined by pruning weights, trunk circumferences, and confirmed by eye. The vineyard was treated uniformly with regard to viticulture, with the exception of crop load, and cluster samples taken on the day of harvest. Berry parameters were measured before clusters were processed for HPLC analyses. Alcoholic fermentation took place using standardized techniques followed by HPLC analyses. Data suggest that balanced vines displaying medium vegetative vigor produce fruit with higher free anthocyanin concentration, more consistent berry parameters, and provide better free anthocyanin extraction rates than vines with either low or higher vigor.

Berry Deformability, Fruit Phenolic Composition, and Extraction into Wine

Tiago Sampaio, M. Carmo Vasconcelos, and James A. Kennedy*
Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331 [james.kennedy@oregonstate.edu]

Along with other fruits, grape berries undergo profound physiological and biochemical changes during fruit ripening, including changes in color, taste, flavor, and firmness. Changes in phenolic composition and extractability into wine as a function of berry deformability were investigated. Pinot noir berries from clusters of clones UCD 17 and UCD 23 were harvested and then sorted into four classes based upon differences in berry deformability. The four classes were designated as plump (berries were firm to the touch), soft (berries were deformable to the touch), wrinkled (berries had wrinkled skins), and raisined (berries resembled moist raisins). Wines were made after adjusting berry volume and sugar concentration so that all treatments were the same. For grape analysis, skin tannin and anthocyanin concentrations in raisined berries were nearly half of that found in soft berries. Raisined and wrinkled berries had higher extractable seed tannin concentrations. A much higher extraction rate of phenolic material present in seeds was also found in the raisined treatment. However, not all extracted seed tannin remained in the wine, as a significant portion was found adsorbed onto skins. Adsorption was particularly apparent in the wrinkled and raisined treatments. Analysis of wine tannin revealed that total tannin concentration and skin tannin proportion were higher in wines made from plump

and soft treatments. Treatment differences in wine polysaccharide content were consistent with those found in grape berries. Informal wine sensory assessment suggests that the observed chemistry differences are related to mouthfeel properties.

Effect of Berry Crushing on Skin and Seed Tannin Extraction during Fermentation and Maceration

Fiorella K. Cerpa-Calderón and **James A. Kennedy***
Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331 [james.kennedy@oregonstate.edu]

Empirical evidence suggests that seed-derived tannins can impart harsh and aggressive tannins to red wine. Minimizing the crushing of fruit prior to fermentation and maceration is generally regarded as effective in reducing the proportion of seed tannin in red wine. To our knowledge there are no studies that provide conclusive evidence that wines produced from uncrushed fruit contain a reduced seed tannin proportion. The purpose of this study was to make this determination. Microfermentations (4 kg fruit) were conducted on *Vitis vinifera* L. cv. Merlot berries that were crushed to varying degrees. Five treatments were established, varying from 0 to 100% crushed berries. Samples were collected throughout fermentation and from the free run and press wine at the time of pressing. Random berries (x 5 replicates) were also collected from the vineyard and were analyzed to determine total potential tannin extraction. Grape and wine tannin extracts were subjected to phloroglucinolysis to determine the amount and proportional extraction of seed and skin tannins. Overall total tannin extraction was consistent with previous studies, increasing with time and crushing. Contrary to conventional wisdom, the highest skin tannin concentration and percent composition was not obtained in the treatments that had a reduced proportion of crushed berries. For all treatments, skin tannin extraction reached a maximum prior to pressing while seed tannins increased throughout maceration. The highest skin tannin proportion was reached on day 10 of maceration with the treatment containing 75% crushed fruit.

Effect of Saignée and Extended Maceration on Wine Phenolic Composition

James Harbertson,* Joshua Maloney, Robert Bertheau, Maria Mireles, and Eric Harwood
Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350 [jfharbertson@wsu.edu]

This study was designed to evaluate the effect of different winemaking practices from Merlot grapes harvested from a single vineyard (5 tons/acre, Columbia River Basin). The experimental treatments and controls were done in duplicate in 10-ton fermentors at a commercial winemaking facility. The experimental winemaking techniques used were extended maceration (20 days), saignée (juice run off), and the necessary controls in attempt to enhance the phenolic composition of the resulting wine. Results showed an enhancement of anthocyanins, polymeric pigments (small and large), and tannins due to altering winemaking practice. We saw no differences in tannins, anthocyanins, or polymeric pigments between a standard water addition

(to compensate for high Brix must) and a saignée that was equivalent to the standard water addition. We did however see significant enhancements in anthocyanins, polymeric pigments (large), and tannins when a larger volume of juice was removed (equal to 10% of the final volume) and extended maceration was employed. The small water addition control that yielded higher ethanol also yielded higher concentrations of polymeric pigments and tannins than the standard water addition control but not enough to be statistically significant. The experiment also showed that the balance of tannin extracted from the skins and seeds was altered depending on the treatment. The standard water addition and low saignée yielded a wine that was balanced with skin and seed tannin while the rest of the treatments favored wines with more seed tannins.

Tannin Partitioning into Extractable and Bound Fractions during Commercial Pinot noir Fermentations

Wynne Peterson-Nedry and Douglas O. Adams*
Department of Viticulture and Enology, University of California, Davis, CA 95616 [doadams@ucdavis.edu]

Tannins (proanthocyanidins) are key components of wine for their influence on astringency, bitterness, ageability, and color stability over time. Understanding the extraction of tannin from grapes into wine is important for forecasting viticultural and winemaking adjustments during harvest and fermentation. In previous work it was noted that not all tannin found in the fruit can be accounted for in the finished wine, suggesting variability of extraction of tannin from berry to bottle. Five commercial Pinot noir lots ranging in fruit fresh weight from 517 to 1,098 kg were sampled in order to account for total tannin present throughout processing. Samples were taken as fruit, at pressing after primary fermentation, and after secondary fermentation. Skins, seeds, mesocarp, pomace, lees, and seeds from the tanks were all ground in liquid nitrogen, extracted with 70% acetone for phenolic analysis by use of protein precipitation, followed by reaction with ferric chloride. The tannin measurements of the postfermentation components were able to account for 48 to 79% of the tannin initially present in the corresponding fruit. An acid-butanol method commonly used for proanthocyanidin analysis was used to assess bound phenolics in all components. Preliminary results indicate that the pomace has approximately 40 to 95% more bound proanthocyanidins than does the fresh fruit. These bound tannins may account for a portion of fruit tannins that are not extracted into wine.

Identification and Toxicity of Microfloral Anthocyanin Metabolites

Sarah C. Forester and Andrew L. Waterhouse*
Department of Viticulture and Enology, University of California, Davis 95616 [alwaterhouse@ucdavis.edu]

Anthocyanins are color compounds in plants that have been shown to prevent and treat many chronic diseases, including colon cancer. Anthocyanins, however, are not well absorbed into the bloodstream and are largely metabolized by microflora to produce phenolic acids and aldehydes in the gastrointestinal tract. The health benefits of consuming foods rich in anthocyanins could be due mostly to antioxidant/

prooxidant mechanisms involving these anthocyanin metabolites. In this study, a Cabernet Sauvignon anthocyanin whole extract containing delphinidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside was incubated in the large intestinal contents of pigs for 0, 0.5, and 6 hr (initial concentrations 0.021, 0.028, 0.061, and 0.298 mM of the anthocyanin compounds, respectively). Anthocyanins and their metabolites were measured by LC-ESI-MS. After 6 hr, anthocyanins were heavily metabolized and no longer detected. After 0.5 and 6 hr, 3-*O*-methylgallate, syringic acid, and 2,4,6-trihydroxybenzaldehyde were identified as anthocyanin metabolites. Based on these and other findings, six anthocyanin metabolites were selected for incubation with caco-2 cells. The effects of these metabolites on cell proliferation, cytotoxicity, and apoptosis are compared to the drug celecoxib. Results from this study indicate that consumption of Cabernet Sauvignon grape anthocyanins could lead to the formation of specific metabolites in the gut, and it is reasonable to suggest that these metabolites offer protection against colon cancer.

Thank you
2008 Best Student Presentation
Award Sponsors

Enology

Lallemand
Petaluma, CA

Viticulture

Sunridge Nurseries
Bakersfield, CA

Thank you for your
continued support

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