

# 62

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## TECHNICAL ABSTRACTS



AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE



## Oral Presentation Abstracts

### Wednesday, June 22

<u>Microbiology</u> .....	53–57
<u>Canopy Management</u> .....	58–61
<u>Pests and Diseases—Part 1</u> .....	62–64
<u>Fermentation</u> .....	65–68
<u>Grape and Wine Phenolics</u> .....	69–71
<u>Pests and Diseases—Part 2</u> .....	72–74

### Thursday, June 23

<u>Sensory</u> .....	75–77
<u>General Viticulture</u> .....	78–80
<u>Water Relations—Part 1</u> .....	81–84
<u>Wine Chemistry—Part 1</u> .....	85–88
<u>Wine Chemistry—Part 2</u> .....	89–91
<u>Water Relations—Part 2</u> .....	92–94

## Poster Presentation Abstracts

### Wednesday, June 22 and Thursday, June 23

<u>Enology</u> .....	95–103
<u>Viticulture</u> .....	104–123
<u>Industry</u> .....	124–129

### Friday, June 24

<u>Grapevine Leafroll and Vitivirus Diseases Seminar</u> .....	130–138
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**NOTE:** pp. 1-50 constituted the program section of the original printed *Program and Technical Abstracts* booklet.

*Abstracts are listed in the approximate presentation order as scheduled in the conference program. Abstracts are those submitted and accepted through the 2011 Call for Abstracts and Call for Posters.*

## Alphabetical Listing of All Authors with Submitted Abstracts

<u>Agüero, C.B.</u> ..... 64	<u>Heinitz, C.</u> ..... 92	<u>Qian, M.C.</u> ..... 98, 113
<u>Alabi, O.J.</u> ..... 74, 135	<u>Henderson, C.M.</u> ..... 90	<u>Ramakrishnan, V.</u> ..... 67
<u>Anderson, M.M.</u> ..... 60	<u>Herr, P.</u> ..... 55	<u>Ramsey, M.S.</u> ..... 56
<u>Arnold, K.L.</u> ..... 132	<u>Heywood, B.</u> ..... 95	<u>Remy, A.</u> ..... 126
<u>Balint, G.</u> ..... 84	<u>Howe, P.A.</u> ..... 103	<u>Reynolds, A.</u> ..... 80
<u>Battany, M.C.</u> ..... 81	<u>Jackowetz, N.</u> ..... 89	<u>Romero, P.</u> ..... 114
<u>Bettiga, L.J.</u> ..... 78	<u>Jones, R.</u> ..... 124	<u>Rwahnih, M.A.</u> ..... 136
<u>Bindon, K.A.</u> ..... 69	<u>Joseph, C.M.L.</u> ..... 53	<u>Salmon, J.M.</u> ..... 65
<u>Bisson, L.F.</u> ..... 65	<u>Jourdes, M.</u> ..... 96	<u>Sanchez Gavito, J.</u> ..... 100
<u>Bondada, B.</u> ..... 104	<u>Keller, M.</u> ..... 59	<u>Scholasch, T.</u> ..... 123, 126
<u>Bonnefoy, C.</u> ..... 79	<u>Kurtural, S.K.</u> ..... 58	<u>Schultz, A.L.</u> ..... 115, 134
<u>Burns, T.</u> ..... 69	<u>Kwasniewski, M.T.</u> ..... 89	<u>Scotti, B.</u> ..... 127
<u>Calvi, B.L.E.</u> ..... 63	<u>Little, H.</u> ..... 124	<u>Shellie, K.</u> ..... 116
<u>Carlos, C.</u> ..... 62	<u>Lund, K.</u> ..... 62	<u>Sim, S.T.</u> ..... 130
<u>Casassa, F.</u> ..... 70	<u>MacDiarmid, R.</u> ..... 130	<u>Smith, R.J.</u> ..... 117
<u>Chittenden, R.</u> ..... 95	<u>Martinson, T.</u> ..... 131	<u>Sommer, S.</u> ..... 54
<u>Collins, T.</u> ..... 71	<u>Matthews, M.A.</u> ..... 61	<u>Song, J.</u> ..... 100, 101, 117
<u>Cousins, P.</u> ..... 104	<u>Mencarelli, F.</u> ..... 81	<u>Specht, G.</u> ..... 128
<u>Creasap Gee, J.</u> ..... 105	<u>Mendez-Costabel, M.</u> ..... 83, 110	<u>Stewart, A.C.</u> ..... 66
<u>Danne K.A.</u> ..... 138	<u>Michaux, S.</u> ..... 77	<u>Striegler, R.K.</u> ..... 118
<u>Delcambre, A.</u> ..... 87	<u>Michel, J.</u> ..... 125	<u>Threlfall, R.T.</u> ..... 75
<u>Dodson, J.C.</u> ..... 72	<u>Monis, J.</u> ..... 133	<u>Utermohlen, V.</u> ..... 75
<u>Favier, M.</u> ..... 55	<u>Montague, T.</u> ..... 82	<u>Vance, A.J.</u> ..... 119
<u>Feng, H.</u> ..... 106	<u>Motosugi, H.</u> ..... 111	<u>Villamor, R.R.</u> ..... 76
<u>Ferrier, J.</u> ..... 88	<u>Murphy, E.E.</u> ..... 97	<u>von Wallbrunn, C.</u> ..... 57
<u>Fort, K.</u> ..... 92	<u>Nail, W.R.</u> ..... 112	<u>Walker, G.A.</u> ..... 68
<u>Fujita, K.</u> ..... 107	<u>Nicholas, K.A.</u> ..... 78	<u>Walker, M.A.</u> ..... 73
<u>Gee, C.T.</u> ..... 108	<u>Nikolantonaki, M.</u> ..... 86	<u>Waterhouse, A.</u> ..... 102
<u>Geller, J.P.</u> ..... 58, 109	<u>Nita, M.</u> ..... 133	<u>Weber, P.</u> ..... 129
<u>Gohil, H.L.</u> ..... 110	<u>Oberholster, A.</u> ..... 91	<u>Wessner, L.F.</u> ..... 120, 121
<u>Gutha, L.R.</u> ..... 106, 136	<u>Osman, F.</u> ..... 137	<u>Wilker, K.L.</u> ..... 103
<u>Harbertson, J.F.</u> ..... 94	<u>Park, S.K.</u> ..... 98	<u>Williams, L.E.</u> ..... 93
<u>Harris, S.A.</u> ..... 85	<u>Pate, D.K.</u> ..... 113	<u>Zhang, Y.</u> ..... 122



Microbiology Session

**Metabolomic Analysis of *Brettanomyces bruxellensis* Strains Grown in Wine and Defined Medium**

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Three *Brettanomyces* strains were grown over 54 days in a defined medium with 10% ethanol and a Cabernet Sauvignon wine. A metabolomics analysis was done at the Metabolomics Core Facility at U.C. Davis by GC-MS-TOF on samples taken at 15 min and 0, 8, and 54 days. Significant changes in metabolic profiles of cells were seen within 15 min of exposure to wine. The comparison between wine and medium grown cells showed a significant increase in metabolites involved in glycerolipid, inositol phosphate, and fatty acid metabolism. Increases in these compounds were not unexpected, as lipid and fatty acids are involved in the stabilization of membranes and inositol phosphate is important in the structure of many secondary messengers in eukaryotic cells. Changes in sugar and nitrogen metabolism were also seen, probably due to the change in available carbon and nitrogen sources between the defined medium and wine. An observed increase in arginine and proline metabolism in wine may be due to the relatively high availability of these amino acids but may also have wider implications as a survival mechanism for *Brettanomyces* in wine. Some evidence indicates that these amino acids are not being used as a source of nitrogen but that arginine is being converted to proline, which may then be used to stabilize proteins. Plants and bacteria use proline to stabilize proteins under salt stress conditions. Strain differences were also apparent in both wine and medium. UCD 2091 gave consistent and significantly different metabolic patterns compared to the two other strains under the same conditions. The other two strains were similar despite their different geographic origins. Metabolic differences in the strains can be attributed to a wide variety of different pathways.

*Funding support:* American Vineyard Foundation and California Competitive Grant Program for Research in Viticulture and Enology.

## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Microbiology Session – CONTINUED

#### **Influence of Grape Composition and Fermentation Strategy on the Formation of Bacterial Off-Flavor in Wine**

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During malolactic fermentation (MLF) the formation of bacterial metabolites might lead to unpleasant aroma characters. The two main groups of considered off-flavors are volatile phenols (e.g., 4-vinylguaiaicol and 4-ethylphenol) and the mousy taint, among others caused by 2-acetyltetrahydropyridine and 2-acetyl-1-pyrroline. Precursors for these substances are phenolic acids for the volatile phenols and amino acids for pyrroline and the pyridines. The aim of this study was to identify factors influencing the off-flavor formation by lactic acid bacteria under pilot-scale winemaking conditions. Circumstances of formation were evaluated under various conditions in viticulture and winemaking practices. The influence of grape rot, must pH, thiamine supplementation, sulfur addition, and lysozyme, as well as fermentation management and MLF strategy, were monitored. Grape varieties used for the experiments were Chardonnay, Pinot blanc, Pinot gris, and Pinot noir of the vintages 2008 and 2009. Off-flavors were analyzed by GC-MS and precursors were measured by HPLC-DAD and GC-MS. The results show only very few cases of mousy taint, mostly after extended maceration prior to alcoholic fermentation. Volatile phenols were found in almost every batch in different concentrations, most of them vinylphenols. Since only few strains of lactic acid bacteria are able to perform the reduction step, only very small concentrations of ethylphenols were produced. A strong correlation between spontaneous or simultaneous MLF and off-flavor production could be observed. If MLF occurs delayed due to lysozyme addition, there is a significantly higher potential for volatile phenol production. Cold soak prior to alcoholic fermentation as well as sluggish metabolism of yeast or bacteria can also lead to higher accumulation of undesired substances. Some grape varieties, such as Chardonnay, seem to favor sluggish fermentation and usually have a higher tendency to develop volatile phenols. A strong relationship between the two factors fermentation rate and off-flavor accumulation could be observed.

*Funding support:* German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn) Project AiF 15833 N.



Microbiology Session – CONTINUED

**A Plasmid Contributing to Wine Adaptation and Technological Properties of *Oenococcus oeni* Strains**

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Lactic acid bacteria responsible for malolactic fermentation in wine mainly belong to the species *Oenococcus oeni*. This species is considered the best adapted to the wine environment, although it comprises a variety of strains that differ in terms of survival to wine stresses and fermentation capacities. Plasmids are an important source of genetic and phenotypic diversity in lactic acid bacteria and occasionally confer adaptive advantages. This study was initiated with the aim to examine their role in *O. oeni* as little is known about *O. oeni* plasmids. During a survey of *O. oeni* strains, we detected a 18-kb plasmid, named pEB03, in a commercial strain. Sequencing of pEB03 revealed that it is a theta-type, nonconjugative plasmid containing 20 putative ORFs. Analysis of 74 *O. oeni* strains by a PCR-based method disclosed pEB03 in 12 additional strains. However, PCR products were slightly larger than expected in five strains, suggesting the presence of a variant form of pEB03. Sequencing of this variant plasmid, named pMF03, revealed roughly the same sequence as pEB03 plus a 4-kb insert. Their similarities suggest that pEB03 is a derivative of pMF03. Interestingly, four of the 12 strains carrying pEB03 or pMF03 were commercial starters (i.e., strains supposedly well-adapted to wine), suggesting that these plasmids may have a technological impact. This was tested using strains containing pEB03 or pMF03 and isogenic derivatives lacking the plasmids in microvinifications. Results showed that the presence of plasmids allowed for a significant reduction of the lag phase and duration of malolactic fermentation. It is concluded that plasmids pEB03/pMF03 play a crucial role in the adaptation and technological properties of *O. oeni* strains.

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**Biogenic Amines in Wine: Evaluation of Various Preventive and Curative Strategies**

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Biogenic amines (BA) are formed at different vinification stages and some of them have toxic effects on organisms. In recent years climate changes favored the increase of must pH, which supports the progeny of undesired bacteria and therefore the danger of increased BA formation. **To influence this wine quality-limiting factor**, preventive and curative strategies have been evaluated. Preventive techniques

## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Microbiology Session – CONTINUED

compared vinification strategies like thermovinification versus skin fermentation and cold soaking combined with flash pasteurization for Pinot noir and Pinot noir Précoce. White wine vinifications with the varieties Chardonnay and Pinot blanc focused on the use of lysozyme and several malolactic fermentation strategies. Another focus was the curative reduction of BA by absorption with bentonite and cell hull preparations. BAs were determined by a modified HPLC method from Pfeiffer (1996). The sensory impact of different strategies was evaluated with descriptive sensory analysis and temporal dominance of sensations (TDS) analysis. Physico-chemical properties of the yeast cell surface were determined with the microbial adhesion to solvent technique and the cell surface hydrophobicity determination. All preventive techniques applied showed positive effects respective to low BA content and sensory perception with more fruitiness and reduced dominance of astringency or coated/anesthetic mouthfeel during TDS. White wines undergoing spontaneous malolactic fermentation were consistently characterized by higher levels of biogenic amines. It could be shown that the positive effect of lysozyme is limited and has to be supported by SO<sub>2</sub> addition. Concerning curative strategies, histamine particularly was reduced significantly by using bentonite. There is considerable variation among different bentonite preparations and applied bentonite concentrations. The absorption effect of BA by yeast cell walls could also be demonstrated. Analysis of variance showed significant differences between yeast cell preparations for each BA. The best absorption results could be observed for phenylethylamine with a maximal depletion rate of 70.6%.

*Funding support:* German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn) Project AiF 15833 N.

### Rapid Identification and Classification of Wine Microbes by MALDI-TOF Mass Spectrometry

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) is an emerging technology for the rapid, low-cost identification of bacterial and yeast strains isolated in clinical settings. To determine if this approach is useful for wine-related microorganisms, we compared results of MALDI–TOF MS patterns to existing molecular fingerprint data of various wine yeasts. Our results show that MALDI–TOF (MS) is a fast and reliable technique that rapidly



### Microbiology Session – CONTINUED

generates a “fingerprint” proteomic profile of various *Brettanomyces* species that readily distinguishes species and, in some cases, strains. Integration of MALDI–TOF MS with existing molecular fingerprint data will provide insight into the true phylogenetic position of these important microbes and sets the stage for testing of MALDI–TOF analyses of microbes directly in wine.

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#### From Grapes to Wine: Monitoring the Biodiversity of Yeasts from Riesling Vines to Wines Using New FT–IR Technology

Daniel Gerhards, Caroline Lehnigk, Nicole Büchl, Mareike Wenning, Siegfried Scherer, and **Christian von Wallbrunn\***

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Wild yeasts derived from vineyard and cellar are important for the wine quality of spontaneously fermented musts. They can lead not only to wines with more distinct aromas but also to wines with off-flavors. It is largely unknown under which circumstances positive or negative aromas unfold. Population dynamics of yeast flora were tracked during spontaneous fermentations, mostly without systematic sensory evaluation of the resulting wines, which does not allow conclusions to be drawn concerning the impact of the flora on wine quality. There are no findings on the composition of yeast flora in German vineyards and their dependence on different soil types (terroirs). In addition, detailed knowledge is lacking on the changes in population structure and assertiveness of certain yeast species and strains during fermentation and their impact on wine aroma. Therefore, the biodiversity of yeasts on Riesling grapes from six vineyards regarding different habitats and soil types in Germany from vintages 2009 and 2010 was analyzed. Yeast diversity was monitored at three different stages before harvest and six times during spontaneous fermentations. At each sampling point, 100 randomized isolated yeasts were identified, ~24,000 isolates in two years. Identification was by FT–IR technology, which allows handling this quantity of isolates and which is based on the absorption of infrared light of different wavelengths by specific cell components. Aroma compounds were detected by GC-MS. The results demonstrated that 12 main yeast species run the first third of the fermentation before it was dominated by *Saccharomyces cerevisiae*. Yeasts arising from the cellar have a stronger influence on the population during fermentation as organisms originated from grapes. Due to the higher throughput of isolates, the FT–IR technology leads to better insight on the yeast biodiversity in vineyard and fermentation.



## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Canopy Management Session

#### Vine Balance Assessment of Pinot Grigio in Southern San Joaquin Valley

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To better understand the optimal canopy management techniques necessary to achieve physiological balance of grapevines, the canopy microclimate of Pinot Grigio was altered through dormant pruning, shoot thinning, and leaf removal in the southern San Joaquin Valley. Twelve canopy levels were imposed with two pruning methods (spur vs. mechanical box-pruning), three shoot density levels (low [23 shoots/m], medium [33 shoots/m], high [49 shoots/m]), and two leaf removal methods (east-side leaf removal, or none) in four randomized complete blocks. Crop load had a direct and positive relationship with shoots exposed per hectare ( $p < 0.0001$ ), number of count shoots per run of canopy ( $p < 0.0001$ ), and yield ( $p < 0.0001$ ). Crop load also had a direct but negative relationship with the distance between shoots ( $p < 0.0001$ ) and vine vigor ( $p < 0.0001$ ). As the distance between shoots increased, count shoots ( $p < 0.0001$ ), cluster number ( $p < 0.0001$ ), shoots exposed per hectare ( $p < 0.0001$ ), and yield declined. The increase in leaf area to fruit ratio was directly proportional with the increase in canopy leaf area ( $p < 0.0001$ ). A leaf area to fruit ratio of 0.8 to 1.3 m<sup>2</sup>/kg, crop load of 7.2 to 9.8 kg/kg, or a canopy leaf area of 14 to 16 m<sup>2</sup> optimized total iron reactive phenolics four months postbottling. These vine balance values are achieved when count shoots are spaced 4.0 cm along the cordon exposing ~90,000 shoots per hectare and leaf removal is conducted on the east side of fruit zone with yield ranging from 22 to 25.4 t/ha. This study provides important information to growers who have adapted to mechanical pruning but not to canopy management practices in the San Joaquin Valley of California.

*Funding support:* American Vineyard Foundation, Bronco Wine Company, Oxbo-Korvan International, West Coast Farming, Viticulture and Enology Research Center.

#### Achieving Vine Balance of Syrah with Mechanical Canopy Management and Regulated Deficit Irrigation

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Canopy microclimate of Syrah 06/1103P grapevines was altered and vines were exposed to regulated deficit irrigation (RDI) with varying severity and timing to mitigate the crop load. Four crop load levels were imposed by dormant pruning to 22 spurs (control) with no further manipulation, and mechanically box-pruning others to 10 cm hedges, and mechanically thinning the canopy to a density of 5 count



### Canopy Management Session – CONTINUED

shoots/30 cm of row (CLL); 7 count shoots/30 cm of row (CLM); or mechanically box-pruning to a 10 cm hedge with no shoot thinning (CLH), respectively. Control vines were irrigated to 70% of ETo from fruit set until harvest (RDIC). Other vines either received 70% of full vine ETo until veraison, after which the rate was cut to 50% of ETo (RDIL), or irrigation was cut to 50% of ETo before veraison (RDIE), but not thereafter. Application of mechanical shoot thinning at stage 17 of the E-L scale by removing 25% of the total shoots exposed with the CLM resulted in exposing about 70,600 shoots per hectare with count shoots spaced about 4.6 cm along the cordon. This exposure translated to about four leaf layers and about 12.6 m<sup>2</sup> of leaf area to ripen the clusters retained on the vines. The leaf area to fruit ratio achieved with the CLM treatment exposed just enough leaf area to ripen the crop level retained on the vines. The combination of the CLM and RDIE treatments decreased the berry weight harvest by 12% without any decrease in harvest weight compared to control, resulting in 25 tons/ha yield. To achieve vine balance with a crop load value of 9.9 kg/kg and a leaf area to fruit ratio of 0.75 m<sup>2</sup>/kg for Syrah in the San Joaquin Valley of California, a combination of CLM and RDIE is needed. The combination of CLM with RDIE results in higher total iron-reactive phenolics, higher tannin concentration, and similar anthocyanin concentration to hand-pruned control with RDIC at harvest. This study provides important information for growers considering mechanizing canopy management operations while scheduling RDI where best results were achieved with the combination of CLM and RDIE treatments to achieve vine balance.

*Funding support:* American Vineyard Foundation, Bronco Wine Company, and West Coast Grape Farming Management.

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### Rootstock Effects on Scion Vigor and Fruit and Wine Composition in a Dry Climate

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A rootstock field trial was conducted in the Yakima Valley of southeastern Washington with three *Vitis vinifera* cultivars (Merlot, Syrah, Chardonnay). Vines were grown on their own roots or field-grafted to the rootstocks 5C, 99R, 140Ru, 1103P, 3309C, and an unnamed rootstock from Cornell University (here termed 101CU). Repeated scion dieback due to cold injury to 99R in late fall led us to abandon this rootstock. Vine vigor and water status, yield formation, and fruit and wine composition were evaluated during three years beginning in the vineyard's ninth year. In 2007, all scion cultivars maintained the highest midday stem water potential on 3309C, but rootstocks did not impact scion water status in 2008 and 2009. Implementation of regulated deficit irrigation seemingly prevented the rootstocks from promoting vine vigor; pruning weights of own-rooted vines were

## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Canopy Management Session – CONTINUED

similar to (Merlot, Syrah) or higher than (Chardonnay) those of grafted vines. Own-rooted Merlot and Chardonnay also grew more shoots than did grafted vines. Own-rooted vines had the highest (Chardonnay), intermediate (Merlot), or lowest (Syrah) yields, while Merlot and Syrah had the highest yields on 3309C. Own-rooted vines had the lowest and 140Ru and 3309C the highest yield:pruning-weight ratio. The rootstocks had only very minor effects on fruit and wine composition. Merlot and Chardonnay fruit and wine from own-rooted vines had more potassium and a somewhat higher pH than did fruit and wine from grafted vines. Wine from own-rooted Merlot and Syrah also had slightly higher tannin concentration than did wine from grafted vines, whereas anthocyanin concentrations were highest in wine from own-rooted vines and vines grafted to 5C and 101CU. Overall, yearly climate variation and scion cultivar, rather than rootstock, dominated the variation in vine performance and fruit and wine composition.

*Funding support:* WSU Agricultural Research Center, Washington Wine Commission, and USDA Northwest Center for Small Fruits Research.

### Interaction of Yield, Pruning Weight and Harvest Date for 20 *Vitis vinifera* Cultivars Grown in the San Joaquin Valley, California

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The viticultural characteristics of 20 *Vitis vinifera* cultivars were described at the Kearney Agriculture Research and Extension Center in Parlier, California (lat. 36.59, long. 119.51). All cultivars were commonly pruned, fertilized, and irrigated. Data were collected for four consecutive years (2007–2010). Mean data from those years showed that yield, pruning weight, and harvest date differed greatly among cultivars. Carmenere and Cinsaut represented the yield extremes with 4.4 and 21.5 kg per vine, respectively. Pruning weight ranged from 0.7 (Petite Verdote) to 2.7 (Carmenere) kg per vine. At the extreme low and high, yield did appear to have an effect on pruning weight, but the two parameters were correlated only at the 90% confidence level ( $r^2 = 0.40$ ). Yield did not have an effect on harvest date (24 Brix) but, again, at the extremes did appear to have an influence. The ratio of yield to pruning weight (Y:PW) is a commonly applied measure to understand the ability of a vine to accumulate berry sugar on a timely basis and sustain desired yield. Data show the Y:PW range across varieties exceeding 10-fold from 23.0 to 1.9. Harvest date was not correlated to Y:PW. Furthermore, Cinsaut and Tempranillo were unable to achieve 24 Brix in any of the trial years and had Y:PW of 23.0 and 8.9, respectively.

*Funding support:* American Vineyard Foundation and Viticultural Consortium West.



### Canopy Management Session – CONTINUED

#### Vine Balance

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“Vine balance” is considered a fundamental principle of winegrape production. I have reviewed the pertinent literature and will show that there is limited experimental research to either demonstrate a balance phenomenon or resolve a biological meaning. It is clear that plants have, or will, establish a balance between root and shoot, because each serves the other necessary nutrients. Whether there is a balance between reproductive and vegetative growth is not established. There is active discussion in plant ecology about the cost of reproduction to subsequent plant growth, but this effect has been difficult to quantify, and it becomes even more difficult to understand and evaluate in perennials like grapevine, which need not produce any fruit in any given year. Thus, the biology of the vine does not lend itself to a simple concept that balances reproductive and vegetative growth. An alternative might be that there is some relationship between the amounts of fruit and of vegetative growth that creates the best winegrapes. This shift to a “balance” based on wine-taster-judgment seems implicit in most uses of vine balance since the mid-1980s, particularly in popular and extension literature, and particularly for Cabernet-type winegrapes. However, a review of the (again limited) empirical work published in reviewed journals shows that it does not support that notion—at least not using the conventional yield:pruning weight metric. Based on the available evidence, there is no actionable meaning of vine balance in grape production, and it has been perhaps premature to take vine balance as an a priori fact that drives a search for itself.

## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Pests and Diseases Session Part 1

#### **New Approaches for Management of European Grapevine Moth, *Lobesia botrana*, in the Douro Wine Region, Portugal**

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The European Grapevine Moth is a key pest of vineyards in the Douro wine region, infesting up to 50% of grapes at harvest. We are examining two main innovative plant protection techniques to reduce pest damage and with minimal environmental impact: pheromone-based mating disruption and an enhanced role of parasitoids. We aim to improve results using each technique and to integrate them into one workable strategy. Mating disruption has been tested since 2000 using ISONET-L dispensers, increasing from an initial 9 ha to 171 ha in 2010. Results have been very promising, but infestation often remains above the tolerance threshold, owing primarily to the high biotic potential of *Lobesia botrana*, its extended life cycle, and the mix of vineyards and untreated habitats common in the Douro region. Currently, we are testing an improved dispenser design. The possibility of manipulating the ecological infrastructure of local farms to improve conservation biological control and to preserve biodiversity is also under study. We hypothesize that we can enhance biological control by arthropod enemies, and by native entomopathogenic fungi, that occur in untreated refugia. Seven species of Hymenopteran parasitoids have been identified to date; the most common are *Elachertus affinis* (Masi), *Brachymeria* sp., *Campoplex capitator* (Aubert), and *Dibrachys cavus* (Förster). Rates of parasitism of *L. botrana* of up to 46.9% can occur in local vineyards. We also plan to develop spatial analysis methods and geographic information systems for improved pest management and to measure biodiversity at the landscape level, relevant to mixed landscapes on highly variable and steep slope terrain.

*Funding support:* Association for the Development of Viticulture in the Douro and QREN (European Fund for Regional Development), through the POFC (Operational Programme for Competitiveness Factors).

#### **A Phenotypic Comparison of Biotype A, Biotype B, and 101-14 Strains of *Phylloxera***

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Phylloxera have been a destructive force in viticulture since their introduction into the wine-producing regions of the world in the mid- to late 1800s. The use of rootstocks bred from American *Vitis* species allowed for the redevelopment of these regions. The extensive use of the rootstock AxR#1 in the 1970s and 80s led



### Pests and Diseases Session Part 1 – CONTINUED

to large-scale decline in California when phylloxera strains overcame its resistance. In an attempt to better understand phylloxera diversity in northern California, four strains were isolated from different vineyards. The Campus strain was collected at the University of California, Davis on own-rooted Chardonnay; the Mendocino strain was collected from AxR#1 in Mendocino County; and the Jordan and Wasson strains were collected from the rootstock 101-14 Mgt in Sonoma County. These strains were maintained on excised root pieces in petri dishes. Excised root pieces were inoculated with 10 to 15 phylloxera eggs and resistance was evaluated in petri dishes by checking every 48 hours to determine the time to first reproduction, number of adults, types of feeding sites, and egg laying rate. The Campus strain acted like a biotype A strain and was unable to feed on AxR#1, while the Mendocino strain acted like a biotype B strain and fed aggressively on AxR#1. Large differences were detected among the Jordan and Wasson (from 101-14 Mgt) strains and the Campus and Mendocino strains. Campus and Mendocino fed aggressively on Colombard roots, while the 101-14 strains did not. The Campus and Mendocino strains did not feed on Riparia Gloire, but the 101-14 types fed aggressively on it. The strains that were able to reproduce on AxR#1 were also able to reproduce on *V. rupestris* Ganzin, one of the parents of AxR#1.

*Funding support:* California Grape Rootstock Improvement Commission, CDFA Improvement Advisory Board, and California Table Grape Commission.

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### Effects of Red Leaf Disease on Cabernet Sauvignon and Mitigation by Crop Reduction and Delayed Harvest

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Grapevines showing foliar symptoms similar to leafroll or corky bark virus infections, but testing negative for the presence of known grape viruses, are often referred to as having red leaf disease. In 2008, a vineyard at the Oakville Experimental Station in Napa Valley, California, was observed to express symptoms fitting this description. The progression of red leaf symptoms was mapped in 2008 and 2009, identifying symptomatic and asymptomatic vines. In 2010, this study aimed, first, to determine if Cabernet Sauvignon grapevines with red leaf disease also expressed delayed maturation symptoms characteristic of the above viruses and, second, to test if those symptoms could be mitigated by crop reduction or delayed harvest. Fruit on vines with red leaf symptoms showed reduced sugar accumulation of 1.9 to 2.7 Brix compared to controls. Reduced sugar accumulation was not significantly mitigated by reducing crop load. By 12 Oct, sugar accumulation in symptomatic vines still lagged behind asymptomatic vines, and the experiment was terminated.

## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Pests and Diseases Session Part 1 – CONTINUED

Photosynthesis, stomatal conductance, and pruning weight were lower for symptomatic vines compared to controls, and severity of red leaf symptoms was directly correlated to a reduction in Brix. Midday leaf water potential, berry weight, juice acidity, and pH were not affected by red leaf symptoms or mitigation treatments.

#### Development of Metabolic Markers Associated with Rootstock-Induced Tolerance to Fanleaf Degeneration

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Fanleaf degeneration is a severe viral disease of grapevines due to its ability to prevent fruit set. It is caused by grapevine fanleaf virus (GFLV) and vectored by the dagger nematode, *Xiphinema index*. The rootstock O39-16, a *Vitis vinifera* x *Muscadina rotundifolia* (VR) hybrid, has successfully been used to control fanleaf for over 15 years. It has excellent resistance to *X. index* feeding and is able to suppress fanleaf symptoms in infected scions. However, it is susceptible to root-knot nematodes and its *V. vinifera* parentage casts doubt on its long-term phyloxera resistance. Efforts to breed alternatives to O39-16 have focused on hybridizing American species of *Vitis* with *M. rotundifolia*. The objective of this project is to accelerate the time required to screen these populations by developing metabolic markers for rootstock-induced fanleaf tolerance. We hypothesize that some graft-transmissible compound from O39-16 roots interferes with GFLV's disruption of fruit set. Cytokinin and metabolite profiling of grapevine xylem sap was performed by GC-TOF/MS and HILIC-LC/MS at the U.C. Davis Metabolomic Core facility. Analysis of 2009-xylem sap collected from healthy and infected Chardonnay grafted on O39-16 and *V. rupestris* St. George showed that isomers of zeatine riboside were the major cytokinin present at fruit set. Zeatine riboside levels in infected O39-16 were higher than in infected St. George, making it a potential biomarker for fanleaf tolerance. In addition, metabolomic profiling analysis of xylem sap samples showed the presence of hundreds of compounds. Eight biomarker candidates were selected after analysis of data based on their abundance in the four treatments at bleeding and fruit set. Testing of xylem sap collected in 2010 is underway. Samples were collected from five VR genotypes with different levels of tolerance. The analysis is focused on cytokinins and the eight biomarker candidates selected in 2009 in order to establish a correlation between biomarker levels and fanleaf tolerance.

*Funding support:* American Vineyard Foundation and California Grape Rootstock Improvement Commission.



### Fermentation Session

#### Comparison of Different Methods for the Determination of Assimilable Nitrogen in Grape Musts

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Different available methods for the determination of assimilable nitrogen in grape musts were studied on 10 musts that were fermented at laboratory scale, in controlled conditions. We first validated the measurement of actual assimilated nitrogen according to the Kjeldahl method as a reference method. Then, the values of assimilable nitrogen obtained by infrared spectroscopy (FTIR), formal titration, orthophthaldehyde (NOPA) + ammonia nitrogen, and amino acids (automatic analyzer) + ammonia nitrogen were compared with actual assimilated nitrogen (difference between the total initial nitrogen concentration and the total residual nitrogen at 80% of fermentation). Results showed that the measurement of amino acids (automatic analyzer) + ammonia nitrogen is the most reliable way to measure assimilable nitrogen in grape musts. But this methodology remains time consuming and quite expensive. FTIR is also a reliable method for the measurement of assimilable nitrogen as it is rapid, convenient, and not sample destructive; it appears as a practical method of assimilable nitrogen measurement on a routine basis. Formol titration and to a lesser extent NOPA + ammonia nitrogen underestimate assimilable nitrogen in grape musts. This study also differentiates between the nitrogen fractions and therefore allows a better understanding of nitrogen assimilation during alcoholic fermentation.

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#### Residual Amino Acid Concentrations after Primary Fermentation: Impact of Arginine:Proline Ratios in Chardonnay Juice

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The effect of four ratios of arginine:proline concentrations (1:1, 1:8, 1:20, and 1:0.25) in YAN normalized Chardonnay juice on the subsequent residual amino acid concentrations after primary fermentation was explored using two yeast strains: EC1118 and L2226. Fermentations achieved dryness uniformly and no variance in replication or strain was observed. Using MANOVA, hierarchical clustering techniques, and PCA, 1:0.25 juices resulted in the significant release of amino acids after primary fermentation. The release of 14 of 22 measured amino acids was significantly impacted by treatment alteration. The varying arginine:proline ratio may affect several biochemical pathways, including permease regulation, epiarginase control, and proline accumulation. Permease expression is tightly regulated by the concentration of both extracellular and intercellular amino acid



## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Fermentation Session – CONTINUED

concentrations. When intercellular levels of both ornithine and arginine are high, as in 1:0.25, epiarginase control may shunt the metabolism of the aforementioned to proline and urea, respectively. Moreover, although proline is not metabolized under anaerobic conditions, proline may act as a membrane-stabilizing component decreasing the release of amino acids when ethanol levels are high. From this study, the ratio of amino acids in juice may alter not only yeast biochemistry but also the resulting amino acid concentrations released after primary fermentation.

### Assessment of Yeast Nutrient Supplements and Residual Nitrogen in Wine

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Nitrogen is a major macronutrient in grape juices and musts that allows *Saccharomyces cerevisiae* to grow, consume sugars, and conduct the alcoholic fermentation. There are two main sources of yeast assimilable nitrogen (YAN):  $\alpha$ -amino acids and ammonium ions. Insufficient amounts of nitrogen, especially in high-Brix juices or musts, can lead to sluggish or stuck fermentations that result in a finished wine with undesirable amounts of residual sugars. Imbalances of amino acids can lead to the evolution of reduced volatile sulfur compounds that are for the most part undesirable in wine. In 2000, we developed recommendations for yeast assimilable nitrogen based on initial sugar content (200, 250, 300 mg/L at 21, 23, 25 Brix, respectively) that have proven successful in most commercial situations. The YAN concentration of juices varies by at least an order of magnitude between varieties, vintages, sites, and so on. We previously reported a range between 40 and 559 mg/L within one vintage on the West Coast of the United States. In addition, there was no correlation between amino and ammonium nitrogen. Robust analytical tools and commercial yeast nutrient supplements are available to measure and correct nitrogen deficiencies. This study assessed the  $\alpha$ -amino-nitrogen contributions of 20 complex yeast foods at manufacturer recommended doses and found them to be modest, ranging from 8 to 24 mg/L. Nitrogen utilization varies greatly among native and commercial yeast strains, and juices from native or hybrid varieties grown in the U.S. Midwest and East can have both relatively low sugar and high nitrogen concentrations at harvest. Subsequently, free residual nitrogen available after alcoholic fermentation can provide a significant nutrient base for spoilage organism, especially surface yeasts and *Brettanomyces* spp. We determined residual YAN in a variety of commercial wines and developed recommendations that ensure the microbial stability of such wines during bulk and bottle aging.



Fermentation Session – CONTINUED

**Adaptive Evolution of Commercial Wine Yeast Strains for Reduced Ethanol Production**

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Extended berry ripening often results in high sugar levels at harvest leading to high ethanol concentrations in wine that can be undesirable. Analysis of the use of *Saccharomyces* for production of biofuels identified two inhibitors, furfural and 5-hydroxymethyl furfural (HMF), that block formation of ethanol. The sensitivity of wine yeast strains to these inhibitors was evaluated and our goal was to use HMF to develop commercial *Saccharomyces* wine strains with reduced yields of ethanol via the process of adaptive evolution. After preliminary screening of 27 wine yeast strains, seven strains were selected for adaptive evolution for 250 generations under a constant pressure of the inhibitor. These strains had three different outcomes in their final ethanol yields (measured by GC-FID) in postadaptive evolution fermentations: one strain displayed a reduction, five displayed slight to modest increase, and finally one displayed no difference in the final ethanol yield as compared to their parent strains. These findings indicate that the mechanism of adapting is different among the yeast strains. The only strain that showed a decrease in the final ethanol yield was also the only one that had no innate resistance to HMF, while the other strains, including the sequenced strains, displayed some level of resistance to HMF. This suggests that the genetic tendency appears to be in the direction of reducing biosynthetic activities that consume reducing power in favor of the reduction of catabolic and environmental aldehydes. Strains representing each group are being evaluated for metabolic basis underlying the adaptive mechanism differences.

*Funding support:* American Vineyard Foundation and California Competitive Grant Program for Research in Viticulture and Enology.

## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Fermentation Session – CONTINUED

#### Alternative Carbon Source Utilization by *Saccharomyces cerevisiae* Wine Isolates Mediated via a Heritable Prion State

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Under normal fermentation conditions, *Saccharomyces cerevisiae* displays an extreme preference for glucose over other carbon sources. Building on work originally presented by Brown and Lindquist (2009), we have found a dominant, stable, transmissible novel prion capable of allowing yeast to overcome glucose-associated repression ([GAR+]) of alternative carbon sources. [GAR+] adaptation is assayed as growth on a complex medium containing glucosamine as a non-hydrolyzable mimetic of glucose and glycerol as an alternative carbon source. With our sequenced yeast strains, we have already seen distinct differences in their ability to adapt to the [GAR+] prion state. We are working to elucidate the genetic basis for differential strength and efficiency of [GAR+] adaptation. Through comparative genomics we have found several amino acid changes that correlate with the specific [GAR+] phenotypes observed. We hope to elucidate the actual prion mechanism, the regulatory network involved, and what specific alleles of particular genes contribute to our observed [GAR+] phenotypes. This prion state is inducible from specific bacteria. Two *Pediococcus* strains isolated from stuck wine fermentations were the strongest inducers of the [GAR+] prion state. We hypothesize that an excreted factor is responsible for inducing the utilization of carbon sources other than glucose and potentially contributing to stuck wine fermentations. With our research we hope to better understand protein-based epigenetic inheritance and the molecular basis for induction of the [GAR+] prion state. Elucidating the mechanism involved in induction and retention of [GAR+] will contribute greatly to our understanding of fermentation dynamics and yeast biology. We hope to develop methods to help wine and other yeast-based industries further control and manage carbon source determination and utilization during fermentations.



Grape and Wine Phenolics Session

**Impact of Malolactic Fermentation on Red Wine Color and Color Stability**

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The effects of malolactic fermentation (MLF) on red wine color and the ability of malolactic bacteria (*Oenococcus oeni*) to degrade compounds important to the development of stable color were studied. In 2009, Pinot noir and Merlot wines were produced, where simultaneous alcoholic and malolactic fermentations were induced in half of the wines. At dryness, all wines were pressed prior to sterile-filtration through 0.45  $\mu\text{m}$  membranes. Wines that had not undergone malolactic fermentation (MLF (-)) were then either (a) inoculated with *O. oeni* (MLF (+)) or (b) pH adjusted to the same pH as MLF (+) wines. All wines were sterile-filtered, bottled, and stored at 13°C for analyses. MLF (+) wines had lower concentrations of acetaldehyde, pyruvic acid, and caftaric acid than MLF (-) wines. MLF (+) wines had significantly lower color and polymeric pigments than MLF (-) wines, while containing significantly higher monomeric anthocyanins. These differences were consistent throughout 12 months of storage and demonstrate that MLF can affect red wine color independent of pH change and that *O. oeni* can impact phenolic and nonphenolic compounds involved in red wine color development. Wines produced in 2010 were used to determine the mechanism by which MLF reduced wine color and polymeric pigment formation. The impact of acetaldehyde and pyruvic acid metabolism was investigated by supplementing MLF (+) wines with acetaldehyde and pyruvic acid to the levels measured in MLF (-) wines. The effect of delaying malolactic fermentation was studied by holding MLF (-) wines at 13°C for 7, 14, 28, and 100 days before inducing MLF (conducted at 21°C). The ability of *O. oeni* to fine color through adsorption was also investigated.

*Funding support:* American Vineyard Foundation.

**Ripening-Induced Changes in Grape Cell Walls Modify Their Interaction with Tannins**

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Cell wall material from Cabernet Sauvignon grape skin and flesh was isolated at different stages of grape maturity in order to determine whether the modification of cell walls during ripening affected their binding properties with tannins. Trends in cell wall affinity and selectivity for tannins were determined using two characterized skin tannins isolated from preveraison (mDP = 18) and ripe (mDP = 33) grapes. Skin and flesh cell wall isolates were combined with tannins at a concentration below saturation, which allowed for the adsorption affinity to be determined

## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Grape and Wine Phenolics Session – CONTINUED

under the same assay conditions. This approach also allowed for selectivity of cell walls for tannins of different molecular mass classes to be determined using gel permeation chromatography. Total insoluble cell wall material per berry increased with grape ripening for two seasons studied, 2009 and 2010. Changes in flesh cell wall affinity for tannins after the onset of ripening were minor and generally showed a higher affinity for tannins of larger molecular mass. On the other hand, skin cell wall affinity for both tannin types increased with the progression of berry ripening. For skin cell wall material from veraison onward, it was found that for both tannins tested that those of higher molecular mass became more strongly adsorbed with the progression of ripening. This effect was enhanced when cell walls were reacted with the ripe tannin isolate. Thus, with the progression of ripening, skin cell wall material had an increasing adsorption affinity for tannin, due to a greater selectivity for higher molecular mass tannin. Furthermore, for skin cell wall isolates there was a positive, significant relationship whereby tannin adsorption for all molecular mass classes increased with berry ripening. The implications of these findings are significant for vinification, since the extraction of tannins from skins of riper grapes may be limited by their stronger affinity for cell walls, in particular tannins of higher molecular mass.

### Unraveling the Effects of Extended Maceration in Merlot Wines with Different Ethanol Levels

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Columbia Valley Merlot harvested from the same block but differing in Brix ( $23.3 \pm 1.4$  vs.  $24.3 \pm 1.0$ ) to target two different ethanol concentrations (EtOH) was processed with two maceration techniques: control (10-day contact) and extended maceration (EM, 30-day contact). The experimental design yielded four treatments with three replicates each. The low Brix treatments had significantly lower EtOH concentration than the high Brix treatments (12.04 and 13.24% v/v, respectively). Anthocyanins and flavonols (HPLC-DAD), tannins (protein precipitation), polymeric pigments (bisulfite bleaching), and color (CIELab) were analyzed during fermentation and postfermentation. Harvest fruit and pomace phenolic composition was also compared. EtOH had no effect on tannins, anthocyanins, polymeric pigments, and color parameters on the finished wines. EM had a higher proportion of large polymeric pigments and higher concentration of iron reactive phenolics than control wines. Significant enhancements in flavonols and tannins were observed during EM in high EtOH wines, but the differences disappeared postmaceration. Tannins dropped 32% (high EtOH) and 33% (low EtOH) two weeks postpress.



### Grape and Wine Phenolics Session – CONTINUED

Control wines maintained higher saturation and red color; however, initial significantly higher color intensities were lost postfermentation. The percentages of pomace anthocyanins recovered ranged from 6 to 29% and were significantly lower in EM treatments. Percentages of pomace flavanols recovered ranged from 61 to 67%, although there was no treatment influence. Variations in ethanol (~1% v/v) had little effect on phenolic extraction and color evolution. EM does not necessarily enhance the extraction of most phenolic compounds of sensory relevance, the only apparent benefit being an increase in polymeric pigments.

*Funding support:* Wine Advisory Committee. Goose Ridge Vineyards provided the grapes.

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### Evaluation of the Relative Impact of Oak Forest of Origin and Grain Tightness on Wine Composition and Sensory Properties

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The objective of this study was to evaluate oak wood with respect to the relative impact of forest of origin and grain tightness on the resulting chemical and sensory properties of wine aged in the barrels made from the wood. Oak stave wood from the Allier, Nevers, and Vosges forests was selected for at least two grain tightness characteristics (open and tight grain defined by number of rings per inch). Four replicate barrels of each combination of forest and grain were made using a consistent barreling protocol. The barrels were filled with Chardonnay juice from one lot; after barrel fermentation and 8 months of aging, the wine was sampled, filtered, and bottled. The wines were analyzed using GC-MS/MS for oak-related volatile compounds; the wines were also analyzed using the Adams-Harbertson assay for polyphenol levels. The wines were evaluated using a sensory technique known as napping, first by a panel of experts and then by a panel of students. It was possible using the chemical analyses to differentiate among some, but not all, combinations of forest and/or grain tightness. Neither the expert panel nor the student panel was able to differentiate among the combinations of forest and grain tightness, although a subset of the most experienced expert tasters performed somewhat better than the panel as a whole.

## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Pests and Diseases Session Part 2

#### Evaluating the Virulence of *Erysiphe necator* Strains with a Leaf Disk Assay

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Powdery mildew is a destructive and widespread fungal disease, which occurs in all the world's viticultural areas. Intensive efforts to identify sources of resistance and the genes that control them have been underway in Europe and Australia. These efforts have resulted in the identification of two resistance genes: *Run1* from *Muscadinia rotundifolia* and *Ren1* from *Vitis vinifera*. There are genetic markers associated with these genes that can be used for marker-assisted selection, but marker-positive seedlings need to be evaluated to confirm marker results and testing of new potential sources of resistance needs to be optimized. An optimized evaluation system should also be able to test and evaluate differences in the aggressivity among strains of the fungus. This study evaluated two French-American hybrids and two Chinese *Vitis* species, as well as a susceptible and resistant control, for resistance mechanisms to strains of powdery mildew isolated from Davis, Monterey, and Paso Robles, California. Leaf disk assay procedures were based on methods developed in the Gubler lab, with modifications to the leaf washing and drying protocols, and the regulation of humidity in culture chambers. The developmental differences among the genotypes were monitored daily by noting and describing spore germination, appressorium, haustorium, and hyphal development. Conidia counts and hyphae diameters were measured at 8 and 14 days after inoculation by counting all conidiophores present or those within a given area. *Vitis davidii* and *V. romanetii* were highly resistant to all of the strains. The resistance of JS 23.416 and Villard blanc varied based on which mildew strain was tested. The virulence of the mildew strains also varied, with the Paso Robles strain being the most aggressive.

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Pests and Diseases Session Part 2 – CONTINUED

**Pierce's Disease Resistant Winegrapes Are Approaching Wine Quality and Field Testing**

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The use of marker-assisted selection (MAS) with DNA markers tightly linked to the Pierce's disease (PD) resistance gene *PdR1* and the acceleration of the seed-to-seed breeding cycle to two years have allowed very rapid progress toward the creation of PD resistant winegrapes. *PdR1*, as single dominant resistance locus originating from *Vitis arizonicalcandicans* b43-17, has been genetically and physically mapped and gene candidates are being tested to proof functionality. In 2009 and 2010, crosses were made to produce seedling populations with *PdR1* resistance and 97% *Vitis vinifera* parentage. MAS was used to select seedlings with the resistance genes and only those with the markers were planted in the field. Crosses from 2009 and 2010 also enlarged the 75% *V. vinifera* populations with PD resistance from *V. arizonicalgirdiana* b42-26 to create an alternative and multigenic source of PD resistance. Selections with *PdR1* at the 94%, 87%, and 75% *vinifera* level are being evaluated at a Beringer Vineyard in Yountville, California, and an additional field plot with 87% and 94% *vinifera* selections was planted in Healdsburg. Several 87% *vinifera* selections were sent to Alabama and Texas for evaluation. Finally, small-scale wine lots were made from five 94% *vinifera* and four 87% *vinifera PdR1* selections. Fruit and juice were evaluated from many other promising progeny at the 94% *vinifera PdR1* level.



## Wednesday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Pests and Diseases Session Part 2 – CONTINUED

#### An Update on Grapevine Leafroll Disease in Washington Vineyards

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Grapevine leafroll disease (GLRD) is a major constraint to sustainability of the grape and wine industry in Washington State. A broad range of GLRD symptoms has been observed in disease-affected grapevines (*Vitis vinifera*) indicating variability in symptoms produced by the disease in different winegrape cultivars grown under cool-climate conditions. Some of these symptoms mimicked those caused by mechanical injury and nutritional disorders, underscoring the need for accurate diagnosis of GLRD using reliable detection methods instead of visual observations alone. Disease surveys during the past five seasons and testing samples for different grapevine viruses revealed the presence of six grapevine leafroll-associated viruses (GLRaV-1, -2, -3, -4, -5, and -9) in several winegrape cultivars. These viruses were found occurring either as single or mixed infections in individual grapevines. Among them, GLRaV-3 was found to be the most widespread in several vineyard blocks. GLRaV-3 was also detected in juice grapes (*Vitis labruscana* Concord and *Vitis labrusca* Niagara), although no symptoms of GLRD were observed in these cultivars. We recently detected GLRaV-1 in Roger's Red, an interspecific hybrid between wild grape (*Vitis californica*, native to northern California) and the *V. vinifera* cultivar Alicante Bouschet, and Claret vine (*V. vinifera* *Purpurea Nana*) showing dull green to scarlet-red colored leaves. We have also detected other grapevine viruses occurring as mixed infections with GLRaVs in individual grapevines. Data on spatial distribution of GLRD indicated clustering of infected vines along rows in vineyard blocks planted with different cultivars. Studies on spatio-temporal spread of GLRD conducted during 2008, 2009, and 2010 seasons showed spread of the disease from heavily infected older blocks to neighboring healthy plantings. Using commercially available pheromones, only the grape mealybug (*Pseudococcus maritimus*) has so far been observed in Washington vineyards.



Sensory Session

Understanding Wine Choice through Consumer Sensory Sensitivity Patterns

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While considerable effort is made to develop wines with specific flavor profiles, little is known about how everyday consumers (in contrast with wine critics and experts) differ in their appreciation of these flavor profiles and the differences in sensory perception that may underlie these preferences. As part of an ongoing study of consumer wine preferences over the past 10 years (Consumer Wine Awards at Lodi, Wine Vision), we are examining the responses of participants in online surveys to determine the relationships of wine type preferences to other non-wine taste-sensitivity-based preferences and behaviors. We are conducting hands-on testing for discrimination thresholds, sensitivity, and identification of 40 aromas and 10 taste solutions with approximately 80 of the survey participants. A preliminary analysis of >900 responses from 2010 has distinguished four different taste sensitivity groups: sweet, hypersensitive, sensitive, and tolerant. The sweet group, accounting for ~20% of the respondents, prefers sweeter wines and white wines and is less likely to drink wine than the other groups. Preliminary data suggest that this less frequent consumption may be due to the difficulty this group experiences, both psychologically and practically, in ordering or obtaining sweet wines in markets where wine experts tout dry wines as more sophisticated and/or appropriate with food. The hypersensitive group (~36% of respondents) prefers light fragrant wines, off-sweet to dry, and will drink red wines but appears to enjoy whites in particular. The sensitive group (~24% of respondents) is open to a wide variety of wines and can appreciate both light and bold wines, while the tolerant group (~20%) gravitates toward bolder wines, Cabernet Sauvignons in particular. The current phase of the study will be completed by summer 2011 and is anticipated to further refine this sensory-based approach to understanding consumer preferences. These taste sensitivity patterns will allow wineries and wine professionals to develop product ideation, flavor development, and marketing strategies.

Using Mixture Designs to Create Red Wine Blends for Consumer Optimization

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An ABCD mixture design was used to optimize 10 *Vitis vinifera* wines (Cabernet Sauvignon, Merlot, and Zinfandel wines plus seven blends) for consumer acceptability, descriptive sensory, and compositional analysis. Consumers (n = 108)

## Thursday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Sensory Session – CONTINUED

judged wine attributes on a 9-point hedonic scale, and data was analyzed using JMP mixture profiler to generate optimized solutions for maximizing individual liking through overall impression. Consumer overall liking scores were modeled to determine an optimized wine blend (44% Cabernet+21% Merlot +35% Zinfandel) across all consumers. However, two segments of consumers were identified after cluster analysis. Segment 1 (n = 60) preferred Cabernet Sauvignon wine and segment 2 (n = 48) preferred Zinfandel wine. Optimized wine blends for each segment were 68% Cabernet+26% Merlot+6% Zinfandel for segment 1 and 27% Cabernet+2% Merlot+71% Zinfandel for segment 2. In addition, a trained descriptive panel (n = 9) rated the 100% Cabernet wine and the 50% Cabernet+50% Merlot wine blend as the highest means for red color intensity, depth of color, herbaceous aroma, spicy aroma, overall flavor intensity, berry flavor, spicy flavor, and mouthfeel. During storage for 12 months (15°C), the seven wine blends exhibited similar compositional changes as their single counterparts with a decrease in total anthocyanins and an increase in polymeric color. Mouthfeel/body was positively correlated ( $r > 0.90$ ) with red color intensity, depth of color, flavor intensity, total anthocyanins, and polymeric color. These results show that identifying niche segments within the population may provide more accurate direction for blending wines to create optimized products for the marketplace.

### Effect of Ethanol and Tannin on the Headspace Volatility of Aroma Compounds in Model Wine

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Wine matrix components may interact with aroma compounds and impact the overall aroma quality of wine. This study investigated the effect of ethanol and tannin on the headspace concentration of eight selected odorants in a full-factorial design. Model solutions were prepared with ethanol (0, 8, 10, 12, 14, and 16% v/v) and tannin (500, 1000, and 1500 mg/L), spiked with fixed concentrations of volatiles normally present in wines. Generally, significant ( $p < 0.05$ ) main effects were observed in odorants for the factors ethanol and tannin. However, in all odorants, ethanol by tannin interaction effects were also significant ( $p < 0.05$ ), indicating that the relative effects of ethanol on odorants were different at varying tannin concentrations and vice versa. Results showed higher odorant concentration observed at lower alcohol concentration and the effect was enhanced with tannin addition. The combined influence of ethanol and tannin most affected eugenol (spicy/clove aroma) followed by 3-methyl-1-butanol (caramel/cooked aroma) and dimethyl disulfide (chemical/sulfury aroma). Sensory evaluation of the same model wines using a trained panel was used to assess the impact of ethanol and tannin



### Sensory Session – CONTINUED

on sensory perception of these eight aroma compounds, with results also indicating that matrix components and their interactions influence the perceived sensory attributes of the wine.

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#### **Effects of Tannin and 4-Ethylphenol and 4-Ethylguaicol on Sensory Properties of Washington Cabernet Sauvignon and Syrah**

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*Brettanomyces bruxellensis* (“Brett”) contamination is viewed as controversial, as in some areas it is considered to be a wine fault. Brett contamination is associated with increased concentrations of 4-ethylphenol (4-EP) and 4-ethylguaicol (4-EG) in the wine. These compounds have been linked with negative sensory properties including increased Band-Aid, barnyard, and medicinal aromas, with the suppression of fruity attributes. Varying ratios of 4-EP to 4-EG have been found in Brett-contaminated wines with an average ratio of 1:8 (4-EG:4-EP) reported. As the presence and ratio of 4-EP to 4-EG may lead to consumer rejection, the first objective of this study was to determine a ratio of 4-EP to 4-EG that would lead to consumer rejection of Washington State Syrah and Cabernet Sauvignon wines. In these wines, two consumer panels (n = 50) were conducted that evaluated three ratios of 4-EP to 4-EG: 1:4 (100 µg/L 4-EG:400 µg/L 4-EP), 1:8 (100 µg/L 4-EG:800 µg/L 4-EP), and 1:12 (100 µg/L 4-EG:1200 µg/L 4-EP). For both wine varieties, a significant decrease in consumer preference was observed at the 1:8 ratio, suggesting consumer preference of 4-EP and 4-EG is between 100 µg/L 4-EG:400 µg/L 4-EP and 100 µg/L 4-EG:80 µg/L 4-EP. As wine composition may influence 4-EG and 4-EP perception in wine, the second objective was to determine the impact of tannin concentration on the wine sensory attributes. Syrah and Cabernet Sauvignon wines were spiked at 1:8 ratio of 4-EG:4-EP, with grape tannin added at 500, 1000, and 1500 mg/L catechin equivalents. Trained panelists (n = 10 and n = 11, respectively) evaluated these wines for medicinal, spicy, leather, and fruity aroma intensity. In Cabernet Sauvignon, increased tannin concentration resulted in increased medicinal and lower fruity aroma intensity ( $p < 0.05$ ). No significant differences were observed in the Syrah treatments. These results suggest that the aroma of Cabernet Sauvignon was more impacted than Syrah by the presence of 4-EP:4-EG.

## Thursday Oral Presentation Abstracts (Research Reports)

2011 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### General Viticulture Session

#### Evaluation of Pinot noir Clonal Selections in the Salinas Valley

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Ten clonal selections of Pinot noir were evaluated for viticultural performance for five years (2005-2009). Pinot noir FPS selections 2A, 4 (Pommard), 13, 23, 44 (reported to be 113), and 46 (reported to be 114) and ENTAV selections 115, 667, 777, and 459 were field budded onto SO4 rootstock planted in 2001 at a vineyard site southwest of Soledad (Arroyo Seco American Viticultural Area). Vines were planted at a row and vine spacing of 2.4 x 1.5 m, trained as unilateral cordons and spur-pruned on a vertical shoot-positioned trellis. Significant differences have been observed in the yield response, with a range of 1.25 kg/vine from high to low yielding selections. Pinot noir selections separated into five groups: 23 being the highest yielding; 2A, 459, and 4 being similar; 115; 667, 114, 13, and 113 being similar; and 777 having the lowest yields. Higher cluster weight was the factor most influencing crop yield. Either more berries per cluster or greater berry weight increased cluster weight. Pruning weights had a range of 0.47 kg/vine from high to low weights. Pinot noir 13 and 777 had the higher pruning weights and 4 had the lowest; the remaining selections were intermediate between the high and low groups. Yield:pruning weight ratios were higher for the more productive selections, ranging from 2.5 (4) to 1.1 (777). There were significant differences among selections in fruit composition at harvest that were independent of crop-load differences.

#### Trends in Climate and Phenological Changes in California and Australia

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Grapevines are sensitive indicators of climate, with the timing of bloom and other seasonal events (phenology) regulated strongly by local interannual patterns in temperature and precipitation. Currently, there is increasing interest in analyzing historical trends of climate and grape phenology, both to understand mechanisms of climate influence on grapevines and to make robust predictions about past and future alterations under climate change. However, such studies to date have been undertaken within specific winegrowing regions, and comparisons between continents, which would suggest how spatially coherent climate tracking by winegrapes is, are lacking. Here we present an analysis of climate and phenological data from winegrowing regions in California and Australia. The most robust records exist for 1980 to the present, but some long-term sites stretch for over 50 years. We compare trends in climate warming between the regions, and correlate these data with bloom, veraison, and harvest dates for the main regional winegrape varieties.



### General Viticulture Session – CONTINUED

Varieties respond in broadly similar ways to climate forcing, but with high variation. We are able to attribute some of this variation to local differences in climate and management between regions, and recent shifts in climate may alter winegrape sensitivity to weather patterns dynamically across space.

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#### Regional and Local Scale Temperature Analysis in Two Winegrowing Regions of California

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The purpose of this study was to determine the role of temperature variations and trends on differences in grapevine phenology within a single winegrowing area. Long-term temperature records from several California weather stations from the USGHCN database were analyzed and their impact on the night cooling index was evaluated. This index plays a predominant role in the synthesis of anthocyanins, one of the primary determinants of wine quality. Results showed a general increase in minimum temperatures with a significant threshold (Pettitt test) between the late 1970s and the early 1980s, depending on location. Minimum temperatures increased from 1.2 to 2°C between the pre- and postthreshold point. During the ripening period (15 Aug-15 Sept), nights became warmer (above 18°C), particularly in the Central Valley. The increase in maximum temperatures was not as evident, with temperatures increasing significantly at some locations and decreasing in certain coastal regions, such as Santa Cruz and Paso Robles. During the summer 2010, 15 data loggers were set up in Lake County and Central Coast vineyards (collaboration between UC Davis and University of Rennes 2 within the framework of the TERVICLIM program). Analysis of temperatures over several months showed marked local contrasts in temperatures, notably within the Red Hills AVA of Lake County, where minimum temperatures could differ by ~15°C between the low and high elevation points (450 to 900 m) within a single vineyard estate. The cooling night index varied from 7.7°C to 9.9°C in the Paso Robles AVA and from 10.5°C to 16.8°C in the Red Hills vineyards. The differences were greater in Red Hills than in Paso Robles due to factors such as onshore flow, elevation, and slope aspect. This spatial variability in temperature within very small geographical areas likely contributes to observed local variations in grapevine phenology.



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