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TECHNICAL ABSTRACTS

62nd ASEV National Conference

June 20–24, 2011

Portola Hotel & Spa and Monterey Conference Center
Monterey, California USA

AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

Oral Presentation Abstracts

Wednesday, June 22

Thursday, June 23

Poster Presentation Abstracts

Wednesday, June 22 and Thursday, June 23

Friday, June 24

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

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)

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-vinylguaiacol 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 adaptative 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.

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

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. Physicochemical 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_2 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

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.

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

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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)

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^2/kg, crop load of 7.2 to 9.8 kg/kg, or a canopy leaf area of 14 to 16 m^2 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^2 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^2/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.

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

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 Verdot) 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)

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 stains 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.

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.

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 *Muscadinia 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 phylloxera 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, orthophtaldehyde (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.

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

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): α-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 α-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)

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 µm 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

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 ± 1.4 vs. 24.3 ± 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.

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.

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 barrelmaking 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)

Pests and Diseases Session Part 2

Evaluating the Virulence of *Eysiphe 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.

Funding support: American Vineyard Foundation and (scholarship funds) H. Baccigaluppi, Robert L. Balzer, Horace O. Lanza, Wine Spectator, Knights of the Vine, Leon Adams, Harold Olmo, and Winkler Fellowship.

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 arizonica/candicans* 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. arizonica/girdiana* 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)

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)

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

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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.

Funding support: Washington State Wine Commission and Northwest Center for Small Fruits Research.

Effects of Tannin and 4-Ethylphenol and 4-Ethylguaiacol 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-ethylguaiacol (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 varietals, 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)

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.

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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.

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.

Thursday Oral Presentation Abstracts (Research Reports)

General Viticulture – CONTINUED

The Terroir of Winter Hardiness: Assessing Spatial Variation in Bud Acclimation, Vine Water Status, and Yield of Riesling and Cabernet franc Using Geomatic Technologies

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Grapevine winter hardiness is a key factor in the vineyard success in many cool-climate wine regions. Winter hardiness, however, may be governed by a myriad of other factors, including soil type, chemical composition, moisture, drainage, vine water status, and yield, that are unique to each site. We hypothesized that winter hardiness would be influenced by vineyard terroir factors and that water-stressed vines would not be as winter hardy as vines with more water available to them. Twelve vineyard blocks (six Riesling and six Cabernet franc) were chosen. Data was collected every 2 weeks (soil moisture, leaf water potential [ψ]), at harvest (yield components, berry composition), and three times during the winter (bud LT50, bud death). Results suggested that factors affecting winter hardiness were vineyard dependent. Spatial trends were clear in each vineyard for soil moisture, leaf ψ, yield components, fruit composition, and LT50. Soil moisture and leaf ψ were correlated spatially across all vineyards. In a low-vigor Cabernet franc vineyard, LT50 was inversely correlated with both yield and leaf ψ, suggesting that both high yield and low leaf ψ (i.e., low water status) might reduce winter hardiness. In a high-vigor Riesling vineyard, yield was inversely correlated to leaf ψ, presumably as a result of high vine size and low fruitfulness in high water status zones. LT50 was inversely correlated with yield and directly correlated with leaf ψ. These preliminary data suggest that there may be a spatial component to winter injury and that this spatial variation might be associated with corresponding spatial variation in soil and vine water status, yield components, or perhaps other unknown variables.

Funding support: Agriculture and Agri-Food Canada and Grape Growers of Ontario.

Water Relations Session Part 1

Determination of the Vineyard Irrigation Crop Coefficients within the Paso Robles Groundwater Basin Area of Concern

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This project quantified the vineyard irrigation crop coefficients corresponding to the fully developed summertime leaf canopies for 84 irrigated vineyards near Paso Robles, California. The vineyards were located in a region east of Paso Robles, which has been experiencing significant declines in the groundwater level due to agricultural and nonagricultural pumping. This groundwater situation has been the impetus for improved locally-specific information on vineyard water requirements to potentially increase irrigation water use efficiency in the area. Field measurements were taken one time at each vineyard location when the leaf canopies were fully developed, between late July and early August 2010, using a solar panel shaded area meter (Paso Panel) to quantify the midday canopy shaded area. The individual shaded area values were converted to irrigation crop coefficients using published relationships. The trellis systems evaluated included vertical shoot-positioned, sprawl, combination vertical shoot-positioned and sprawl (shoots positioned only on one side), Geneva double curtain/quadrilateral, and lyre. The average crop coefficient value for all sites was 0.53. The differences in the average crop coefficient values between the different trellis systems were not as large as the range of crop coefficient values observed within each individual trellis category. This indicates that there is considerable potential for improving the accuracy of crop coefficients by measuring them on an individual site basis rather than by relying on generic crop coefficient values based solely on trellis type.

Funding support: American Vineyard Foundation.

Time of Vine Defoliation Affects the Nebbiolo Grape during Postharvest Dehydration for Wine Production

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Nebbiolo vines were defoliated after fruit set (FS) and at bunch closure (BC), thus permitting bunch exposure to the sun. After harvest, grapes were subjected to dehydration at three temperatures (10, 20, and 30°C), 50% RH, and continuous 1 m/sec air flow until reaching a weight loss of ~20%. This weight loss permitted sugar concentrations to reach 28 to 29 Brix, which is the level for the production of highly prized Sfurzat wine. At 30°C, defoliation did not affect the rate of weight loss, while at 20°C there was significant difference between nondefoliated and defoliated grapes. Defoliation delayed weight loss above all in the FS sample.

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Total color difference changed significantly in all the samples and the blue color tended to increase in defoliated grape. The FS sample showed less respiration rate than nondefoliated and BC samples. Malic acid diminished during the dehydration but no difference was observed among the samples. K+ ions increased significantly during dehydration especially in the FS sample and FAN decreased in the other two samples during dehydration but not in FS. Total polyphenols increased during dehydration significantly but without difference among the samples. Anthocyanins did not change. Lypooxygenase activity, as an index of water stress, increased at 20% weight loss and was lower in the FS sample. Proline increased significantly in the nondefoliated sample in accord with the weight loss pattern.

Physiology of Cabernet Sauvignon Grapevines Grafted to Different Rootstocks on the Texas High Plains

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On the Texas High Plains grapevines are subjected to climatic extremes. To assist growers when selecting varieties and rootstocks adapted to the region, this study investigated rootstock effects on physiology of field-grown Cabernet Sauvignon grapevines. Research took place at a commercial vineyard near Lubbock (research supported by the Texas Department of Agriculture). *Vitis vinifera* Cabernet Sauvignon scions grafted to nine rootstocks were used: 1103P (*V. berlandieri* x *V. rupestris*), 3309C (*V. riparia* x *V. rupestris*), 420A (*V. berlandieri* x *V. riparia*), 5BB (*V. berlandieri* x *V. riparia*), Freedom (1613C x *V. champini*), Harmony (1613C x *V. champini*), Riparia (*V. riparia*), SO4 (*V. berlandieri* x *V. riparia*), and own rooted. Vines were planted spring 2008 in a completely randomized block design. For each rootstock and scion combination, there were a total of 25 plants in each block. All plants received similar irrigation volume. Budbreak data was collected during spring 2010, and on four occasions during the growing season physiological data were measured with a LI-COR 6400. Means were exposed to ANOVA. If differences were detected, then means were separated by Fisher's least significance difference procedure. Budbreak occurred later in the growing season for scions grafted to 5BB and SO4 rootstocks. Physiological data (photosynthetic rate, stomatal conductance, water use efficiency, leaf to air vapor pressure deficit) indicated physiological differences between Cabernet Sauvignon scion and rootstock combinations were not consistent and favored no particular rootstock. Because this is a young vineyard (and to give further insight of adaptability of these scions to the West Texas High Plains), physiological and production (fruit production, fruit quality, pruning weights, etc.) data will be collected during the next several growing seasons.

Water Relations Session Part 1 – CONTINUED

Remote Sensing of Real-Time Water Use in Commercial Vineyards

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Irrigation management is one of the most important drivers of fruit and wine quality in California. Traditionally, irrigation scheduling has been done either by tracking indicators of vine water status over time or by estimating the crop's evapotranspiration during a given interval. Plant-based measurements, such as midday leaf water potential and shoot growth, can vary significantly within a particular field and are difficult to replicate in large-scale operations. Accurate estimates of vine water status for an entire field are therefore very difficult to obtain. Crop coefficients are needed in order to obtain estimates of crop evapotranspiration and are usually a linear function of the shaded area measured beneath the canopy in fully irrigated vines. These crop coefficients also vary significantly within and between vineyards, as differences in soil texture or irrigation management effect canopy size, therefore modifying vine water use. The goal of this study was to compare estimates of vine water use provided by a surface energy balance model with field measurements taken in two commercial vineyards of Cabernet Sauvignon in the Central Valley of California. Satellite-based data was provided by SEBAL North America. Stem water potential, leaf stomatal conductance, and soil moisture readings were taken on the day of image acquisition in both vineyards. Field measurements of shaded area were used to calculate crop coefficients and vine water use in several plots within each vineyard, and then compared to data recorded by satellite-based sensors along with ground-based weather data to solve the energy balance at the earth's surface, yielding spatially distributed estimates of actual evapotranspiration. Both estimates were not significantly different, especially before veraison, when vines did not show symptoms of water stress. Results show that the surface energy balance model can be used to estimate water use of fully irrigated vines, providing a coverage of spatial variability that would be very difficult to achieve with current indexes such as leaf water potential or soil moisture readings.

Thursday Oral Presentation Abstracts (Research Reports)

Water Relations Session Part 1 – CONTINUED

Impact of Water Status on Abscisic Acid and Its Catabolite Profiles in Leaves and Berries from *Vitis vinifera* L. Chardonnay in a Cool Climate

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To understand the relationship among soil and plant water status, plant physiology, and the hormonal profiles associated with it, abscisic acid (ABA) and its catabolites (phaseic acid, PA; dihydrophaseic acid, DPA; 7-hydroxy-ABA, 7-OH-ABA; 8-hydroxy-ABA; neophaseic acid, and abscisic acid glucose ester, ABA-GE) were analyzed in leaves and berries from *Vitis vinifera* L. Chardonnay under different water status treatments. The method used liquid chromatography with ion trap combined with electrospray ionization–mass spectrometry. The irrigation trial was carried out during the 2006 and 2007 growing seasons, on a Chardonnay commercial block located in Niagara-on-the-Lake, Ontario, Canada. The field trial consisted of seven treatments: a control (nonirrigated) and three water levels (100, 50, and 25% of crop evapotranspiration) that were combined with two timings of irrigation imposition (fruit set and veraison). ABA, ABA-GE, DPA, and 7-OH-ABA were found in detectable amounts at all sampling dates, while PA was found in small amounts only at some sampling dates. The hormonal profile was different in each year of the experiment, which indicated a direct relationship between ABA and climatic factors. The hormonal profile showed less temporal variation in 2006 than in 2007. The ABA concentration varied during the season, showing a high correlation with soil and plant water status. ABA followed different catabolic pathways depending on the plant water status. ABA was likely catabolized by conjugation to form ABA-GE in treatments under higher levels of water deficit, while in treatments with high water status, the oxidation pathway leading to DPA or PA was likely preferred. ABA and ABA-GE were the most important compounds, which correlates well with soil moisture and Ts during the whole growing season. The ABA and ABA-GE concentrations in the berries at harvest showed a high relationship with the water status during the season.

Wine Chemistry Session Part 1

Three Approaches for Quantification of 3-Isobutyl-2-Hydroxypyrazine, a Key Intermediate in IBMP Metabolism

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3-Isobutyl-2-hydroxypyrazine (IBHP) is thought to be a key intermediate in both the biosynthesis and degradation of the herbaceous smelling 3-isobutyl-2-methoxypyrazine (IBMP), but quantitative methods for IBHP analysis are not widely reported. As IBHP is generally in the same concentration range as IBMP, appropriate quantification methods should achieve low ng/L detection thresholds using modest sample sizes. We previously reported a two-dimensional GC (GCxGC)-TOF-MS approach for IBHP analysis, but this approach required large sample sizes, and GCxGC is not available in most labs. We explored three approaches for quantification of IBHP in winegrapes (*Vitis vinifera*): gas chromatography/electron impact–mass spectrometry (GC/EI-MS), gas chromatography/positive chemical ionization–mass spectrometry (GC/CI-MS), and liquid chromatography/electrospray ionization–tandem mass spectrometry (LC/ESI-MS/MS). Grape juice at pH 2.0 was loaded onto Plexa PCX solid-phase extraction cartridges, washed with 5% v/v methanol:water (pH 2.0), and IBHP was eluted with 2% v/v NH4OH in 4:1 ethyl acetate:DCM solvent. Limits of detection for GC/EI-MS were very poor due to high interferences and poor response of IBHP. GC/CI-MS with methanol CI reagent greatly reduced background noise and increased response such that detection limits of ~20 ng/L in juice could be achieved. Similar limits of detection were achievable with LC/ESI-MS/MS in positive ion mode with selected reaction monitoring (SRM or MRM) analysis. To compensate for sample losses during extraction, an isotopically labeled internal standard, 4[H2]-IBHP, was prepared. The recovery yield and suppression effect from matrices were also assessed along with traditional MRM-based quantitative analysis of both IBHP and IBMP. Both GC/CI-MS and LC/ESI-MS/MS are appropriate for trace level quantification of IBHP. We are using these methods to compare IBHP and IBMP concentrations during the growing season.

Wine Chemistry Session Part 1 – CONTINUED

Some Insights into the Effect of Polyphenols and Antioxidants on Sauvignon blanc Wine Varietal Thiol Compounds

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Sauvignon blanc wines are known to exhibit volatile thiols with distinctive passionfruit and box-wood aroma. 3-Sulfanylhexanol (3SH) with grapefruit odor is described as key molecule contributor of Sauvignon blanc wines typicality. Volatile thiols are particularly susceptible to degradation during wine aging and improving their stability is seen as a key to retaining fresh, fruity character over time. Polyphenol oxidation has been associated with the loss of varietal volatile thiols, and the role of antioxidants such as sulfur dioxide or glutathione may be critical to varietal aroma stability. The reaction rate between 3SH and the major white wine phenolic compounds (caftaric acid, (+)-catechin, (-)-epicatechin) was established in winelike medium. Caftaric acid was slightly reactive with 3SH, contrary to flavan-3-ols (+)-catechin and (-)-epicatechin. Among phenolic compounds, flavan-3-ols appeared to be the highest traps for volatile thiols during wine aging. Moreover, partial least square regression was applied to associate the Sauvignon blanc wine typicality with their levels in some antioxidant (sulfur dioxide, glutathione) or pro-oxidant (flavan-3-ols, caftaric acid, dissolved oxygen) factors. The statistical model explained more than 61% of the total variance and confirmed complex multivariate relationships between chemicals and typicality. In general, typicality was positively correlated with varietal thiols, glutathione, and caftaric acid and negatively correlated to compounds showing less favorable odors, such as ethyl-3-sulfanylpropionate or ethyl-2-sulfanylpropionate and flavan-3-ols (assayed in dry white wines at concentrations ranging from 0.1–44 mg/L). Additionally, the direct effect of flavan-3-ols, (+)-catechin or (-)-epicatechin, to varietal thiol degradation was demonstrated during bottle aging. The presence of 25 mg/L of (+)-catechin or (-)-epicatechin accelerated (30%) 3SH degradation after 10 months. Their effect was amplified twice when wine was sealed with a synthetic in comparison with a screwcap closure. In both cases, the direct effect of (+)-catechin and (-)-epicatechin addition to 3SH decrease was observed once glutathione, sulfur dioxide, and dissolved oxygen were almost completely consumed (after 10–12 months). During the first months of conservation of the wines in bottles, concentrations in 3SH decreased regularly and independently of the presence of the flavan-3-ols. After the glutathione was completely consumed in the wine, the chemical oxidation of the flavan-3-ols in relation with closure oxygen permeability appeared to impact directly on the degradation of volatile thiols.

Wine Chemistry Session Part 1 – CONTINUED

Collection of High-Resolution Isotopic Patterns of Grape Seed Tannins by UHPLC-Q-TOF-MS

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The proanthocyanidins or condensed tannins are polymers of flavan-3-ol units, such as (-)-epicatechin or (+)-catechin. Numerous studies in HPLC-MS have been performed to analyze proanthocyanidins in wine and grape seed. However, there has been a lack of data concerning the higher oligomers and polymers, which are important in establishing characteristic mouthfeel in a particular red wine variety or origin. We examined the optimization of MS fragmentation voltage and high mass isotopic pattern resolution in order to discriminate single and multicharged proanthocyanidin oligomers in red wine by UHPLC-Q-TOF-MS. Pinot noir grapes were sampled in 2010 (Okanagan Valley, BC). Grape seeds were first manually separated and extracted in acetone/water (70/30) to give a tannin crude extract. The samples (grape seed and red wine) were analyzed by ESI-Q-TOF-MS. Different parameters such as fragmentor voltages (100 to 300 V), collision energy (slope of 3 V/100 and offset of 5 V), and positive or negative ionization mode were investigated. First, mass spectrometer parameter effects (fragmentor voltage, ionization mode, and collision energy) were studied on grape seeds and red wine samples. In both ionization modes (negative and positive), the signal of molecular ions decreased with the degree of polymerization. The optimization of fragmentor voltage allows better detection of higher oligomer compounds in wine. Dimers of (-)-epicatechin were observed at 100 V, but trimers were observed at 200 V. Higher fragmentation voltage did not increase the response significantly, thus the fragmentor was set at 200 V. Next, the sensitivity and high resolution of the mass spectrometer allowed the detection of doubly and triply charged ions of overlapping oligomeric proanthocyanidins in the sample. The high resolution isotopic patterns of molecular ions showed the presence of singly charged trimer, tetramer, and pentamer and, respectively, doubly charged hexamer, octamer, and decamer in grape seed. Deconvolution of the charged series should allow quantification of each oligomer of proanthocyanidin in the future by using appropriate synthetic standards. The ratio of each oligomer could potentially be correlated to unique taste or mouthfeel characteristics of a wine or allow discrimination among wine varieties. Further work is underway to optimize the detection of oligomer ions by studying solvent and pH effects.

Thursday Oral Presentation Abstracts (Research Reports)

Wine Chemistry Session Part 1 – CONTINUED

Impact of Bottle Closures on the Shelf Life of Commercial Wines

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Previous research has shown that different bottle closures can impact the quality of bottled wines over time (shelf life), with oxygen having a significant role. Several questions remain about the interaction of the wine matrix, the closure, and bottling conditions. We bottled three commercial wines (one light and fruity Sauvignon blanc, one early-to-bottle Merlot, and one barrel-aged Merlot) during commercial bottling runs using several closure variables and including some novel variants. The wines were then monitored as they aged over the course of three years for the Sauvignon blanc and five years for the Merlots. We were also able to quantify the oxygen ingress rates for many of these closures and estimate ingress rates for the others. Our results show that closures with high oxygen ingress caused wines to age rapidly. Low-ingress closures resulted in wines that aged more slowly. Score (preference) as measured by a highly calibrated small panel did not differ significantly across the different closure types. Instead, ingress rates were a greater determining factor. The impact of nitrogen-sparging red wine screwcap bottle headspace was less than expected, compared with previous studies using noncommercial single-head cappers. The appearance of reduction in a few treatments occurred early and did not change significantly over time.

Wine Chemistry Session Part 2

Using a Simple Assay for Elemental Sulfur Residues to Evaluate Effects of Viticultural and Prefermentation Practices

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Hydrogen sulfide (H_2S) production during fermentation can result from several causes, including elemental sulfur (S^0) residues on grapes. S^0 is a common control for powdery mildew, but studies of S^0 persistence are limited by an absence of routine analytical approaches. We have developed a rapid, inexpensive method for quantifying S^0 with sensitivity comparable to GC-MS or HPLC and appropriate for a vineyard or small winery. Following dispersion in polyethylene glycol and buffering, a 1 to 5 g sample was treated with dithiothreitol (DTT) to reduce S^0 to H_2S, which was in turn quantified by a commercial sulfide detection tube. The method has a limit of detection of 0.01 µg S^0/g sample with good linearity from 0.03 to 100 µg S^0/g (R^2 = 0.994, mean CV = 6.4%). Mean recovery of a S^0 containing fungicide was 84.7% with a mean CV of 10.4%. Total sample analysis time was <30 min and the cost was $5 to 10.00 per sample. With this method, field trials were conducted to understand S^0 persistence following late-season sprays stopped 2, 4, or 6 weeks before harvest. Cessation of spraying 2 weeks preharvest resulted in S^0 residues well above concentrations reported to yield excess H_2S during fermentation (range = 14.3–42.9 µg/g). Cessation of sulfur sprays 6 weeks before harvest resulted in still detectable residues (3.2–7.6 µg/g). Must clarification reduced residues by >95%, although increased H_2S production was still observed in one of the treatments where S^0 had been applied 2 weeks before harvest. Finally, late season sprayed Cabernet franc was vinified with varying degrees of skin contact (whole cluster pressed, crushed/destemmed, 24 hr cold skin contact, 7 day maceration, 14 day maceration). Despite different concentrations of S^0 residue in the treatments, H_2S production (91–112 µg/L) during vinification was not significantly different. However, H_2S in wines following storage was significantly higher in treatments with longer skin contact.

Funding support: Federal Formula Funds.

Management of SO_2 Binding Compounds during Vinification to Increase SO_2 Efficacy

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The use of sulfites (SO_2) during winemaking has been practiced for centuries. Sulfites are added for their antioxidant, antienzymatic, and antibacterial properties and help protect wine quality over time. Wines with little or no SO_2 additions, such

Wine Chemistry Session Part 2 – CONTINUED

as organic, are becoming popular among consumers who are sensitive or believe they are sensitive to sulfites. To address these consumer preferences and to abide by SO_2 addition regulations, management of SO_2 binding compounds is crucial. SO_2 binding compounds can bind to SO_2 and dramatically decrease its preservative actions, thus necessitating increased SO_2 addition to achieve the active "free" or unbound form of SO_2. There are numerous SO_2 binding compounds in wine, although this work focused on major binders, including acetaldehyde, pyruvate, α-ketoglutarate, acetoin, galacturonic acid, and glucose. To better understand the quantities of SO_2 binding compounds in wine, a survey was conducted and 240 commercial wines from New York State (NYS) were analyzed using a precolumn derivatization method with HPLC separation and UV detection. Acetaldehyde, pyruvate, and glucose showed higher relative concentrations in white wines, whereas α-ketoglutarate and galacturonic acid were higher in red wines. A follow-up study was then conducted to trace acetaldehyde throughout the vinification process in 10 commercial wineries in NYS and identify critical control points for its production. Results suggest that once wine is removed from residual yeast or bacterial lees there is a higher probability of acetaldehyde accumulation. Furthermore, the effects of 12 commercial strains of *Oenococcus oeni* were evaluated for their impact on SO_2 binder levels during MLF. *O. oeni* were found to metabolize both acetaldehyde and pyruvate extensively, while across most fermentations acetoin, α-ketoglutarate, and galacturonic acid concentrations remained largely unchanged. This research provides new insights into the frequency of SO_2 binding compounds in commercial wines and will help to identify strategies for their management to increase the efficacy of SO_2 during vinification.

Identifying the Critical Lipid Components of Yeast Biomembranes That Contribute to a Successful Wine Fermentation

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Ethanol and other fermentation products have previously been implicated in stuck and sluggish wine fermentations. It is likely that one factor that determines ethanol tolerance in yeast is the composition of the cell membrane. Understanding the mechanism by which ethanol tolerance is conferred has been hampered by a lack of lipid structural data from fermenting yeast. While numerous studies support the hypothesis that the membrane composition of *Saccharomyces* sp. is critical in determining ethanol tolerance and fermentation progression, differing experimental conditions between studies have made it difficult to draw definitive conclusions. Furthermore, the methods of analysis used in these studies did not preserve a full range of lipid structural data. Small-scale fermentations were carried out with 25 industrial yeast strains, including numerous wine yeast strains that exhibited mark-

Wine Chemistry Session Part 2 – CONTINUED

edly different sugar utilization and ethanol tolerance characteristics in a defined synthetic grape juice. Lipids were extracted from yeast at numerous fermentation time points using a modified Folch method developed by our group. Phospholipids were quantitatively analyzed via a normal phase liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) method that we developed. Flow injection analysis by atmospheric pressure chemical ionization–mass spectrometry (APCI-MS) was used to quantitatively determine sterol concentration in yeast lipid extracts. Lipid structure and identity were confirmed using tandem mass spectrometry and fragmentation databases developed by our group. Overall, more than 60 phospholipids and sterols are being quantified. Multivariate statistical analysis correlating lipid membrane components to fermentation kinetic parameters is examined in the context of the relationship between lipid composition and successful completion of fermentation. Results will be used to guide process or strain modification to increase the ethanol tolerance of wine yeast strains with other favorable flavor or quality attributes.

Funding support: USDA-CREES, American Vineyard Foundation, and California Competitive Grant Program for Research in Viticulture and Enology.

Influence of Tannin Addition, Microoxygenation, and Wood on Red Wine Phenol Composition and Aging

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The addition of commercial tannins and microoxygenation treatments are widely used techniques in red winemaking. Different commercial tannin preparations containing hydrolysable tannins, condensed tannins, or mixtures of these two are available on the market. Tannin suppliers report color enhancement, oxidative protection, and flavor and mouthfeel improvement. The few research studies that have been published have shown limited influence by tannin addition on wine color and phenol composition. Hydrolysable tannins extracted into the wine by the use of wood alternatives or barrel maturation is proposed to similarly stabilize and protect wine color. Barrel maturation has been shown to improve the color of red wine. Microoxygenation mimics the slow uptake of oxygen in wine during barrel maturation. Several studies claim that the effect of microoxygenation on wine color is similar to that which occurs during maturation in oak barrels, although only one study actually compared microoxygenation and barrel maturation. In addition, the joint effect of microoxygenation and wood treatments has been investigated extensively, with most investigations confirming a positive effect on wine color and phenol composition.

Water Relations Session Part 2

Chloride Exclusion Patterns in Six Grapevine Populations

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Rootstock breeding for improved salt tolerance will depend on the accurate characterization of hybrid populations segregating for chloride exclusion. Previous studies indicate that salt tolerance may be inherited either qualitatively or quantitatively, depending on the parentage of the individuals being tested. In addition, data from greenhouse assays have not consistently reflected field performance data with mature vines, leading to further confusion. We developed a greenhouse-based assay that rapidly characterizes the capacity for chloride exclusion by a given genotype and that has repeatedly yielded results similar to those obtained in the field. This assay was applied to six populations: (1) a selfed population of *Vitis vinifera* cv. Colombard, (2) *V. champinii* cv. Ramsey x *V. riparia* cv. Riparia Gloire, (3) Ramsey x Colombard, (4) Ramsey x *V. rupestris* cv. St. George, (5) *V. vinifera* x St. George, and (6) *V. vinifera* x *V. berlandieri*. Results revealed a surprising capacity for chloride exclusion in some progeny of the selfed Colombard, which seemed to have a quantitative inheritance for chloride exclusion. Also surprising was the response of Ramsey x Riparia Gloire, which did not have greatly increased tolerance when compared to the selfed Colombard population. Ramsey x Colombard, Ramsey x St. George, and *V. vinifera* x St. George performed as expected, with a quantitative inheritance and with the best exclusion within the *V. vinifera* x St. George population. Most individuals of the *V. vinifera* x *V. berlandieri* population had strong chloride exclusion, which may be due to a dominant, single, and fixed allele for chloride exclusion in the *V. berlandieri* parent. Genetic markers linked to chloride exclusion will be pursued in these populations.

Funding support: California Grape Rootstock Improvement Commission, CDFA Improvement Advisory Board, California Table Grape Commission, and (scholarship funds) UC Davis-Jastro/Shields, Wine Spectator, Horace O. Lanza, and David E. Gallo Educational Enhancement Fund.

Germplasm Screen for Chloride Exclusion in Wild Grapevines

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As water becomes less available for irrigation of arid climate vineyards, the lack of rootstock choices to tolerate saline conditions will become an increasing concern. Few salt-tolerant rootstocks are available, and these often have other undesirable growth characteristics. Some grapevines have the ability to exclude chloride ions from the shoot, minimizing the damage from salinity. We hypothesized that wild grapevines growing in arid and saline areas may have evolved chloride exclusion to

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survive under harsh conditions. We used a recently developed greenhouse screening method to evaluate 113 wild *Vitis* genotypes collected from the southwest United States for their ability to exclude chloride, including *V. arizonica, V. girdiana, V. berlandieri, V. rupestris,* and several other native species and hybrids. Many genotypes displayed lower chloride uptake than *V. champinii* cv. Ramsey, the typical salt-tolerant benchmark for rootstocks, and some displayed values statistically similar to the unsalted controls. The full results of this screen will be reported, along with plans to further investigate the genetic basis and evolutionary implications of the chloride exclusion trait.

Funding support: California Grape Rootstock Improvement Commission, CDFA Improvement Advisory Board, California Table Grape Commission, and (scholarship funds) UC Davis-Jastro/Shields, Wine Spectator, Horace O. Lanza, and David E. Gallo Educational Enhancement Fund.

Effects of Applied Water Amounts on N and K Nutrition of Thompson Seedless Grapevines Grown in the San Joaquin Valley

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The effect of soil water balance on N and K availability could be as important as the direct effects of water stress on plant growth. The purpose of this study was to determine the effects of applied water amounts on grapevine mineral nutrition. Thompson Seedless grapevines were irrigated at various fractions of evapotranspiration (ET_c) measured with a weighing lysimeter. Irrigation amounts were 0.2, 0.6, 1.0, and 1.4 of ET_c with treatments imposed for four years. Petioles were sampled at anthesis and vines were harvested several times during the third year of the study. Dry biomass, N and K of the clusters, leaves, and stems (main axis of the shoot) were quantified. Petiole nitrate-N and K concentrations measured at anthesis were not significantly different among irrigation treatments any year measured and values of both were within the adequate range for Thompson Seedless. The concentration of N in the leaves and clusters as a function of irrigation treatment did not differ significantly on any sample date during the third growing season. Leaf dry weight and N per unit leaf area were significantly greater for vines in the 0.2 irrigation treatment compared to vines irrigated at the 1.0 and 1.4 levels when measured after June. Vines irrigated at the 1.4 level had significantly greater values of K (% dry weight basis) in the leaves, stems, and clusters at the end of the growing season compared to the other three irrigation treatments. The greatest values of N and K in the vines were equivalent to 107 and 140 kg ha^{-1}, respectively, while those in the 0.2 treatment were 44 and 39 kg ha^{-1}, respectively. Under the conditions of this study, N nutrition was not affected by deficit irrigation, while overirrigating increased the K concentration in all vine organs.

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Effect of Deficit Irrigation Timing and Vintage on Anthocyanins and Tannins in Cabernet Sauvignon Grapes and Wine

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Regulated deficit irrigation was applied to a 30-year-old own-rooted Cabernet Sauvignon vineyard in Mattawa, Washington, to evaluate the effect of altering the timing and duration of the deficit on grape and wine phenolic composition over three vintages (2008 to 2010). Evapotranspiration (ET) was used to estimate full-vine ET and manage the irrigation applications. The experimental deficits applied were early season, veraison, full season, and none (control). The control received 70% ET, whereas all of the treatments were 35% ET during the deficit. The experiment was carried out in a randomized complete block using north-south row orientation with four repetitions per treatment. Wines were made in duplicate by combining two of the vineyard replicates at a commercial winery (1,542 kg). Analysis of variance showed significant treatment and vintage effects for many of the compounds measured. In each vintage, the full season deficit and early season deficits had significantly lower berry weights than the other treatments. However, only the full season deficit had significantly greater anthocyanins and skin tannins on a fresh weight basis than the other treatments for all vintages. Our phenological and weather data suggests that the duration and rate of degree-day accumulation during the biosynthetic periods for skin tannins and anthocyanins significantly impact their final concentrations at harvest. Wines made from the deficit irrigation treatments showed similar trends to fruit composition. Sensory evaluation of 2008 wines showed significant differences among the treatments, with distinct color and astringency profiles for each of the experimental treatments.

Funding support: Washington Wine Advisory Committee, Washington Wine Commission, and WSU Agricultural Research Center.

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Comparison of Three Different Analysis Methods for Tannin in New Zealand Red Wines

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A representative sample of 12 New Zealand (Hawkes Bay) red wines from several grape varieties and recent vintages were analyzed to compare the relative tannin, total phenolic, and total anthocyanin concentrations, using two established methods and one new method. The recently developed spectral calibration method implemented in the Tannin Web Portal of the Australian Wine Research Institute was compared with the methyl cellulose precipitable (MCP) tannin method and the Adams-Harbertson (A-H) protein precipitable tannin method. We observed strong correlations with the comparisons of relative tannin concentrations analyzed by the three different methods. Correlation between the MCP tannin assay reference values from the Australian and New Zealand laboratories was strong ($r^2 = 0.9181$), supporting inter-laboratory robustness. The relationship between the newly implemented predictive method and the reference data showed strong correlation ($r^2 = 0.8756$) between the values generated in the New Zealand laboratory for both the MCP reference method and the predictive calibration, thereby giving confidence in using the rapid method. Inter-laboratory comparison of the reference methods (A-H and MCP), which is relevant for comparisons between labs running the different methods, was strongly correlated ($r^2 = 0.8905$) and linear, but the slope highlighted the previously observed difference in magnitude of results from the two assays (A-H values < MCP values). The rapid method and the A-H reference method values were also compared and, again, correlation was strong ($r^2 = 0.8383$), linear, and the magnitude difference was observed. Results give confidence in using the AWRI Tannin Web Portal as a tool for wineries to determine tannin concentrations in fermenting and macerating red wines much more rapidly than has been previously possible.

Funding support: TechNZ. Hawkes Bay Wine Growers Association Inc.

Effect of Tannin Additions Postfermentation with Gelatin Remediation in Paso Robles Cabernet Sauvignon

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The resultant color of a Cabernet Sauvignon wine connotes overall quality. Therefore, it is in the best interests of a winery to produce a well-balanced wine, containing the deepest color possible, using the least amount of additives. This experiment studied the effects of adding ColorPro, VRSupra, and Gelatin Extra No.1 to Caber-

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net Sauvignon fruit from Paso Robles to determine if anthocyanin and tannin levels changed with respect to the aforementioned supplements. To increase color and tannin extraction, ColorPro was added to the grape must prior to fermentation. A tannin powder, VRSupra, was added later in the winemaking process to reduce the bitterness and astringency obtained as a by-product of the enzyme addition. It is our presumption that the more tannin added throughout the winemaking process, the less gelatin remediation is needed on the wine prior to bottling. Three different tanks of Cabernet Sauvignon were monitored and analyzed. Varying rates of ColorPro were added at the hopper (0, 60, and 100 mL/ton). Postfermentation, four neutral American barrels were filled with free-run wine from each tank. Different rates of VRSupra tannin were added to the four barrels from each tank (0, 10, 20, 40 g/hL). The Adams assay was used to quantify amounts of total phenols, anthocyanin, tannin, and long and short polymeric pigments. Results suggest that there are no significant differences in the resultant stable color of the wines, but tannin levels were influenced by the addition of ColorPro, as well as VRSupra, determined by the Harbertson-Adams assay.

Identification and Quantification of Ellagitannins and Their Derivatives during Red Wine Aging (Oak Barrels/Oak Chips)

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Red wine aging is generally performed in oak barrels. During this long-term contact with oak wood, red wine extracts a wide variety of substances, such as aromatic molecules and ellagitannins. Vescalagin and castalagin were the first ellagitannins to be isolated and characterized after their isolation 30 years ago from *Castanea* (chestnut) and *Quercus* (oak) woody species. Six additional ellagitannins were later isolated from *Quercus* and *Castanea* hardwood species. It has been recently shown that ellagitannins from oak and flavanols from grapes can form flavano-ellagitannins during wine aging in oak barrels. These flavano-ellagitannins are hybrid tannins with an ellagitannin moiety connected to a flavanol moiety via a carbon-carbon linkage. Recently, the hemisynthesis of these flavano-ellagitannins has been achieved between vescalagin and catechin/epicatechin in organic acidic media and in wine model solution. We first monitored the extraction rate of the ellagitannins by HPLC-UV-MS on several wines obtained from different grape types and in different aging conditions (oak barrels/stainless-steel tanks with oak chips). During wine aging, the concentration of ellagitannins increased regularly to reach a maximum at 3 to 4 months in oak barrels, whereas this maximum concentration was

observed after 1.5 to 2 months in stainless-steel tanks with oak chips. Moreover, we also monitored for the first time the formation rates of the flavano-ellagitannins in red wine during aging in oak barrels and in stainless-steel tanks with oak chips. These flavano-ellagitannins were found in red wines aged in oak barrels one month after barreling, whereas they were found in stainless-steel tanks with oak chips after only two weeks. However, the total amount of the flavano-ellagitannins was two times lower for the red wine aged in stainless-steel tanks with oak chips than for the red wine aged in oak barrels.

Novel Regulation of Individual *HXT* Genes Could Hold the Key to Understanding Stuck Fermentations

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Stuck fermentations are a persistent risk in winemaking. Understanding how yeast detect and consume low levels of sugar at the end of fermentation will help winemakers avoid this common problem. The *HXT* gene family has been well-characterized as encoding the hexose transport proteins responsible for bringing sugar into the yeast cell, and loss of activity of these proteins can impact fermentation progression. The low sugar-sensing pathway is mediated by the membrane protein Snf3p that functions to detect low levels of sugar outside the cell and sustain sugar metabolism. When *snf3* is mutated the high-affinity *HXT* genes are not expressed and residual sugar is not efficiently utilized. This can be overcome and growth restored by overexpression of the kinase Sks1p. Sks1p acts specifically to increase expression of *HXT2*. Sks1p phosphorylates the negative regulators of the *HXT2* gene, but we have shown this does not result in their degradation. Instead Sks1 acts via an unconventional regulatory mechanism. We have isolated mutants that impact Sks1-mediated growth and regulation using a novel selection mechanism based on the toxicity of transport of 2-deoxyglucose. Several genes were identified that when mutated prevented Sks1p from inducing expression of *HXT2*. To define the mechanisms of action of these genes and their potential role in reduction of fermentation rate, combinations of these mutations and other known mutations affecting *HXT* gene regulation were generated. Some of these mutations seem to function by decreasing overall growth properties of the cells and define interacting elements of *HXT* gene regulation.

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Determination of Optimum Concentrations of DAP Additions to Reduce the Production of H_2S during Wine Fermentation

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Must is frequently supplemented with *di*-ammonium phosphate (DAP) to prevent nitrogen deficiencies or imbalances, which are important risk factors for sluggish or stuck fermentations as well as the separate issue of excess hydrogen sulfide (H_2S) production. However, fermentation problems frequently persist despite the addition of sufficient DAP to the nitrogen-deficient must. Moreover, excessive addition of nitrogen may lead to elevated formation of ethyl carbamate (EC), which has been shown to be carcinogenic in animals. Most wine-producing countries strictly regulate maximum permissible additions of DAP. In this study, H_2S production by three wine yeast strains was measured during experimental fermentations with 10 increasing concentrations of DAP, ranging from 24 to 240 mg/L as ammonium, to a nitrogen-deficient juice. These three yeast strains were high (Rhone 2323 and CM #47) and moderate (Enoferm CSM) producers selected from a total of 85 wine yeast strains. Production of H_2S during fermentation was monitored daily using the Sulfur Stick, a sulfide-detecting tube. Concentrations of H_2S produced were not directly and inversely proportional to juices having elevated DAP additions, and production patterns were different for each yeast strain. In general, the highest DAP addition showed the lowest H_2S production by the three strains tested with a range of 7.9 to 264.0 mg/L. However, one strain (Enoferm CSM) produced very low levels of H_2S (11.3 mg/L), even though the DAP addition was only 120 mg/L, suggesting that each yeast strain has an optimal requirement for DAP additions in order to minimize H_2S production. This study demonstrates the importance of establishing optimum, yeast strain-dependent nitrogen additions for reducing H_2S production in nitrogen-deficient juices or musts at the laboratory-scale fermentation prior to large-scale fermentations.

Comparison of Screwcap and Cork Closures on Oxygen Permeability and Flavor Development during Postbottle Aging

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Bottled wine is a dynamic system and a proper wine bottle closure will allow a dynamic and healthy gas exchange between the wine and the air. The amount of oxygen ingress through the wine closure can directly affect wine aging and flavor development. Natural cork (up and down), synthetic closures, and screwcaps with Saran-Tin, Saranex, and low-density polyethylene (LDPE) liners were investigated on both Pinot noir and Chardonnay wines over three years of storage. For Char-

donnay wine, the LDPE screwcap gave the highest dissolved oxygen, lowest free SO_2, and highest absorbance at 420 nm. The Saran-Tin screwcap gave the lowest dissolved oxygen. Similar trends were observed for Pinot noir wines, with Saran-Tin screwcap giving the lowest dissolved O_2 and highest free SO_2 and total SO_2. Hydrogen sulfide, methanethiol (MeSH), dimethyl sulfide (DMS), methylthiol acetate (MeSOAc), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) were extracted using a Carboxen/PDMS SPME fiber and were quantified by gas chromatography-pulsed flame photometric detection using methyl ethyl sulfide and isopropyl sulfide as internal standards. Reduction of H_2S, methanethiol, and methylthiol acetate was observed during the three-year aging process for both wines. The reduction of H_2S and MeSH correlated well with oxygen permeability of the closure and dissolved oxygen, and their concentration decreased most in wines with LDPE screwcap and synthetic closures, while the decrease of MeSOAc was independent of closure type. For both wines, we did not detect any elevated sulfur compound for any screwcap closure. No DMDS or DMTS was detected in any of the experimental wines. Potential volatile sulfur conversion was also investigated in synthetic wine at 4°C, 40°C, and 50°C for four months, with eight sulfur-containing compounds: MeSH, DMDS, DMS, MeSOAc, methionol, methionine, cysteine and glutathione. DMDS is very stable in synthetic wine during storage, while DMS can be converted to MeSOAc and DMDS. MeSH can be easily converted to DMDS and DMTS. MeSOAc is stable at 4°C, but some conversions to MeSH and DMDS were observed at elevated temperature. Methionol can be easily converted to DMDS. Cysteine can generate H_2S, while glutathione can generate DMS.

Funding support: American Vineyard Foundation.

Sensory Space of Reposado Tequilas

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There is no scientific literature on the sensory attributes of Reposado Tequilas from Mexico. The only published work on this distilled spirit is on the chemical volatile profile of tequilas. We were interested to determine whether the price of Reposado Tequila affected its sensory attributes and its position on the sensory space. Twenty Reposado Tequilas ranging in price from US$10 to US$50 for a 750 mL bottle were evaluated by 11 panelists in triplicate by descriptive analysis using 11 sensory attributes. The same tequilas were also subjected to a napping exercise using 16 panelists who evaluated the tequilas in triplicate. The napping data indicated that 12 descriptors (fruity, citrus, floral, soapy, agave, lime, smoke, wet hay, chemical, Play Doh, woody, and dried fruit) completely describe the Reposado Tequila napping sensory space. The descriptive analysis sensory space was described by the following attributes: Play Doh, lemon, smoky oak, tea-tree oil, herbal, fruity, floral,

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orange, soapy, honey, and alcohol. The two sensory spaces were quite similar and again it seems as if napping could be substituted for descriptive analysis. The tequilas did not only separate by price in the sensory space (although some of the less expensive tequilas were quite different from the more expensive ones), but, more interestingly and quite unexpectedly, tequilas made by the same house were more similar to one another regardless of price. For example, Centenario (US$26) and Herradura (US$38) were similar as were Cazadores (US$33) and Corzo (US$46).

Sensory Descriptive Analysis and Napping of Gins from Four Countries

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The scientific literature on the sensory attributes of gins is sparse. We were interested in whether gins from four countries (England, Germany, France, and the United States) differed sensorially and/or based on their volatile profiles. Twenty-five gins were evaluated by 15 panelists in triplicate by descriptive analysis using 12 sensory attributes. These gins were also evaluated in duplicate by 12 panelists using a relatively new sensory technique called napping. Additionally, gins were analyzed by SPME-HS GC to determine their volatile profiles. The descriptive and napping data sets were compared and essentially described the same sensory space. It may be possible to substitute napping for descriptive analysis in cases where there are clear-cut differences in the sample sensory space. However, the volatile profiles were not very similar to the sensory space, indicating that individual chemical compounds do not describe sensory responses but that matrix effects are more important. The gins from France and Germany were similar to one another (they were high in alcohol and caramel intensities relative to the other gins) and very different than the gins from England and the United States. Some of the English (for example, Bombay and Beefeater) and American gins were very similar to each other (Bellringer and Lords) and others were quite different (New Amsterdam and Booths from the United States; Juniper Organic and Plymouth from England).

Effect of Ultraviolet and Visible Radiation on Volatile Composition of Pinot noir Wine

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Sunlight, especially ultraviolet (UV) radiation, can affect plant metabolism and change grape berry composition, such as anthocyanins, total phenols, carotenoids, and amino acid contents. However, the effect of UV radiation on flavor

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compounds of wine is unknown. To understand the influence of UV and visible radiation on flavor composition of wine, Pinot noir grapevines grown in Australian were subjected to four canopy treatments: (1) dense canopy-shaded bunch (SB), (2) leaf removed canopy-exposed bunch (EB), (3) vines covered with polycarbonate screen, blocking UV radiation (UV-), and (4) vines covered with acrylic screen, transmitting radiation(UV+). Ten bunch replicates were sampled from each field treatment and fermented. The aroma compounds were determined using stir bar sorptive extraction-gas chromatography-mass spectrometry (SBSE-GC-MS). The results showed that the volatile compounds responded differently to UV and visible radiation. The straight chain ethyl esters as well as methyl hexanoate and hexyl acetate had the highest concentrations in wine from the UV-treated grapes, whereas the concentrations of ethyl 2-methyl butanoate and ethyl 2-phenylacetate were the highest in wine from dense canopy-shaded bunches. The UV+ and EB treatments led to higher levels of terpenols such as citronellol, nerol, and geraniol in wine, with the exception of linalool, which was highest in the BS treatment. For C_{13}-norisoprenoids, both the UV- and UV+ treatments had an increase in β-damascenone, with UV- having a significantly higher increase. Vitispirane and β-ionone were not affected by the treatments. In addition, the UV+ treatment significantly increased 2-phenylethanol, while significantly decreased octanoic and decanoic acids.

Funding support: China Scholarship Council.

Comparison of Volatile Compounds in Pinot noir Wines from Different Vine Vigor Zones

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Vine vigor directly affects grape and vine physiology, which could affect the composition of secondary metabolites. The objective of this research was to investigate the vine vigor on volatile composition of final wines. Four vigor zones (ultra low, low, mediate, and high vigor) of Pinot noir vine were determined by infrared aerial photography, and wines were produced separately from those grapes harvested in each zone. Stir bar sorptive extraction-gas chromatography-mass spectrometry (SBSE-GC-MS) was used for determining the flavor compounds in wines. Statistical analysis showed that vine vigor significantly affected aroma composition in wines. Lower vine vigor resulted in higher concentrations of esters, including ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methyl butanoate, ethyl 3-methylbutanoate, ethyl pentanoate, hexyl acetate,

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ethyl heptanoate, ethyl 2-phenylacetate, and ethyl vanillate. The highest concentrations were also observed for linalool, nerol, and geraniol from the ultra-low-vigor treatment, although the concentration of citronellol was higher under mediate and higher vigor. In addition, low-vigor density resulted in higher concentrations of C_{13}-norisoprenoids such as vitispirane and β-ionone, but inconsistent results were observed for β-damascenone.

Funding support: China Scholarship Council.

Quinone Products in Wine Oxidation

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Quinones are reactive chemical species that are formed in abundance during the oxidation of wine. They are known to strongly react with nucleophilic compounds, but to date there has been little work aimed at understanding their interaction with wine-relevant nucleophiles. However, it is hypothesized that quinone–nucleophile reactions may be central to controlling one aspect of wine aging chemistry, and more specifically, these reactions may govern changes in wine characteristics due to oxygen introduction and subsequent reaction. Here, the quinone of 4-methylcatechol was prepared using an oxidizing reagent that yields quinone in a few seconds. Then a series of nucleophilic substances were added to the solution and the loss of quinone was followed by loss of characteristic quinone UV absorbance. Reaction rates varied by over 100,000 from the fastest to the slowest, indicating significant differences in reactivity. The reaction products must be fully characterized to assess the reaction pathways, and these model reactions must be followed by tests in actual wines to see if these reaction kinetics can be reproduced under practical conditions. The large range in reaction rates suggest that wine oxidation could be better controlled with specific treatments.

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Effects of Tannin Levels on Color Stability in Norton Wine

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Proper color development in red wines is thought to require tannin to anthocyanin ratios greater than two. However, supporting research is limited. Norton wines typically have tannin to anthocyanin ratios less than one due to moderate amounts of tannin and high anthocyanin levels. An experiment was conducted to determine how the addition of tannin would affect long-term color stability and the formation of polymeric pigments in Norton wine. Commercial grape seed tannin was added at 500 and 1000 mg/L after pressing. Wines were kept in glass containers with periodic splash racking to incorporate oxygen during the aging process of three years. Tannin additions did not result in significant differences in color (absorbance at 420, 520, and 620 nm) or the fraction of color due to copigmentation. Greater tannin levels resulted in higher ratios of large to small polymeric pigments but did not result in significantly higher amounts of polymeric pigments. Both small and large polymeric pigments increased for all wines throughout the aging process. Increasing tannin levels in the wines resulted in less total red pigments and free anthocyanins. All wines retained relatively high levels of free anthocyanins and absorbance at 520 nm. These results suggest that not all wines require tannin to anthocyanin ratios greater than two for acceptable color stability during extended aging.

Funding support: Missouri Department of Agriculture.

Evaluation of Analytical Methods and Winery Laboratory Proficiency from 1999–2009

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Winery analytical proficiency covering 33 rounds of testing (66 wines) from the Collaborative Testing Services was evaluated. This 10-year range of data shows industry performance over time and across wine matrixes. Removal of outliers allowed a realistic evaluation of actual industry performance for various analytes and methods. Overall average results, standard deviations, and resulting coefficients of variation indicate industry proficiency (CV of <5%) in only four common analytes: titratable acidity, alcohol, density, and pH. Other analytes such as malic acid, residual sugar (separated into the reducing sugars or glucose and fructose in 2004), volatile acidity, and both free and total SO_2 have coefficients of variation ranging from 10.8% to 24.9%. Relative method biases, interaction of matrix on method performance, and changes in methods used over the past 10 years show potential areas for improvement.

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Viticulture

A Mechanistic View of Grapevine Vigor-Xylem Anatomy and Hydraulics

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To gain an understanding of the mechanistic basis of vigor phenomenon in grapevines, the Merlot cultivar was induced to develop different shoot lengths, an index of vigor by pruning the vines to a range of bud numbers. Physiological and anatomical measurements were recorded at specific phenological events in all vigor levels. In a given shoot, the successive internode lengths and the corresponding leaf area increased linearly at the distal end, whereas the opposite occurred at the proximal end. Vessel size, vessel density, and radial sectors bordered by xylem parenchyma were smaller at distal ends than at proximal ends; the opposite was true for vessel density. Vessel number, total vessel area, hydraulic conductivity, and percent sapwood area decreased acropetally in a shoot. Shoot components, stomatal conductance, cluster weight, and nutrient levels increased with shoot length, whereas Brix decreased with cluster number per shoot. Vessel number per cross-sectional area, sapwood area, vessel diameter, vessel area, and hydraulic conductance increased with shoot length, whereas vessel density decreased with shoot length. This study showed that vigor and xylic features were interrelated.

Funding support: Northwest Center for Small Fruits Research.

Rootstock Hybrid Seedling Progenies of Rubired Winegrape Segregate for Red Leaf Color Potential

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In order to develop improved grapevine rootstocks with red leaves as morphological markers, two elite nematode resistant rootstock selections ((*Vitis berlandieri* x *V. riparia*) x *V. biformis*) were crossed with Rubired winegrape (*Vitis vinifera* hybrid). Rubired vines have red leaves in the late growing season and fall, which distinguish them from most other grape varieties. The red color of Rubired leaves is thought to be inherited as a single dominant allele from its ancestor, Teinturier du Cher, through Alicante Bouschet and Alicante Ganzin, but the transmission of red leaf color from Rubired is not described. Seedlings from controlled pollinations were grown in a greenhouse and fully expanded leaves were sampled. Leaf pieces (0.9 cm diameter) from each seedling were surface sterilized, then floated abaxially on a solution of 100 g/L sucrose in water. Leaf color was observed after 10 days in an illuminated incubator (16 hr light, 8 hours dark, 26°C). Each seedling genotype

was classified as either "red leaf" or "green leaf" according to leaf disk color. Both rootstock parents are classified as green leaf, while Rubired is classified as red leaf. In one population, there were 164 red leaf seedlings and 166 green leaf seedlings. In the other population, there were 152 red leaf seedlings and 171 green leaf seedlings. In both cases the segregation of the red leaf phenotype is statistically consistent (by chi-square test) with the conditioning of that trait by a single dominant allele for which Rubired is heterozygous, with 1:1 red leaf:green leaf segregation in both populations.

Funding sources: USDA ARS, American Vineyard Foundation, California Table Grape Commission, California Raisin Marketing Board, and California Grape Rootstock Improvement Commission.

Development of Berry Growth Curves in Hybrid and *Vitis vinifera* Grapes to Enable Crop Estimation and Crop Adjustment in New York and Missouri

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Seasonal berry growth curve information has been useful for midseason crop estimation and adjustment in Concord grapevine production. Similar information could potentially be used in interspecific hybrid and *Vitis vinifera* grape production; however, little information exists on seasonal berry weight in hybrid and *V. vinifera* varieties. The purpose of this project was to establish berry weight and diameter curves for Concord and common hybrid and *Vitis vinifera* grape varieties in New York and Missouri to enable growers to estimate and potentially thin their crops accurately and nondestructively. In the Lake Erie and Finger Lakes Regions of New York and in Missouri, individual berries from 15 different varieties in the three distinct regions were collected throughout the 2010 season from 15 to 20 days after bloom through harvest. Individual berry diameters and weights were measured and recorded. Sample sizes ranged from 100 to 200 berries per sample, for berry weight measurements, and sample sizes for the berry diameter measurements ranged from 30 to 100 berries. For the 2010 growing season, there were strong positive, linear relationships between berry weight and berry diameter for the sampled cultivars ($p < 0.001$). This indicates that a nondestructive sampling and crop estimation method may be feasible by measuring berry diameters in the field to estimate berry weights.

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Studies on Impacts of Grapevine Leafroll Disease on an Own-Rooted Winegrape Cultivar

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We conducted studies in an own-rooted Merlot block in eastern Washington to measure impacts of grapevine leafroll disease (GLRD) on vine performance, fruit yield, and berry and wine quality. Grapevines were identified in a commercial vineyard in such a way that individual vines exhibiting typical GLRD symptoms and tested positive for *Grapevine leafroll-associated virus 3* were adjacent to healthy grapevines in a given row to minimize errors in sampling and experimental results due to variations in growing conditions. Data collected during the 2010 season showed differences in leaf photosynthesis and chlorophyll fluorescence between GLRD-affected and unaffected leaves only after veraison. An analysis of macro- and micronutrients in leaf samples collected at two critical time points (midbloom and late veraison) from GLRD-affected and unaffected grapevines showed that they were generally within the normal range and indicated no significant differences. Fruit maturity indices (soluble solids and fruit acidity) and total anthocyanins measured at various stages of berry development showed significant differences between berries produced by GLRD-affected and unaffected grapevines. Cumulative fresh fruit yield per vine measured at the time of commercial harvest showed significant yield reduction in GLRD-affected grapevines. Small-lot wines made from grapes harvested from GLRD-affected Merlot grapevines showed significantly less amounts of pigments (anthocyanins, small- and large-polymeric pigments), tannins, and alcohol than in wine made from fruit harvested from unaffected grapevines. These results clearly demonstrated that GLRD affects vine performance and negatively impacts fruit and wine quality in own-rooted Merlot grapevines grown under cool-climate conditions of eastern Washington State.

Cover-Crop Management on Free and Bound Volatiles of cv. Pinot noir Grapes

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Vineyard alleyway management is an important aspect of growing healthy grapevines and producing high-quality fruit. Very little research has reported the impact of cover-crop management on grape volatile composition. It is our hypothesis that cover-crop management will affect vine growth and could result in changes in

grape volatile composition. The impacts of vineyard alleyway cover-crop management on Pinot noir grape volatile compounds and volatile precursors were investigated for four consecutive years. Grapes harvested from three treatments—clean cultivated (C), alternate alleyway cover/tillage (A), and solid grass cover (S)—were harvested and analyzed from 2007 to 2010. Free volatile aroma compounds in grapes were analyzed using the stir bar sorptive extraction (SBSE)-GC-MS method. The bound precursors were isolated with C18 chromatography and hydrolyzed by glycosidase under acidic conditions. The released volatiles were analyzed by SBSE-GC-MS. Cover-crop management treatments did not differ in volatile and precursor composition in 2007; however, treatments influenced grape volatile and precursor compositions in 2008, 2009, and 2010 vintages. Although there were wide variations in fruit from treatments, consistent trends were observed among three vintage years. Grapes grown under alternate cover crop (A) showed the highest amounts of total bound terpene alcohols (e.g., linalool oxide, 4-terpineol, α-terpineol, and linalool) and C_{13}-norisoprenoids (e.g., β-damascenone, vitispirane, and 3-hydroxy-β-damascenone). Grapes grown under solid grass cover (S) showed the lowest amount of bound form of total terpene alcohol and C_{13}-norisoprenoids. For both 2009 and 2010, clean cover gave the highest concentration of free hexanal and t-2-hexenal, which is typically associated with less ripe fruit.

Funding support: Oregon Wine Board.

Pyrosequencing Analyses of Fungal Community in Japanese Vineyard Soils

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Soil microbes play an important role in the management of soil environment. In vineyard soils, however, the overall microbial community structure has yet to be unraveled. To understand the microbial community in vineyard soil, we surveyed comprehensively microbial communities in Japanese vineyard soils by using a culture-independent molecular approach. First, DNA was directly extracted from the soil samples. Then, the fungal internal transcribed spacer 1 (ITS 1) region or the bacterial 16S ribosomal DNA (rDNA) region was PCR-amplified and sequencing of cloned fragment. We identified 681 fungal clone sequences and 1076 bacterial clone sequences, and the results suggested that *Ascomycota* is the dominant group in the fungal community, whereas *Proteobacterium* and *Acidobacterium* are the dominant groups in the bacterial community. Moreover, the microbial community structures in the vineyard soils were extremely complex, suggesting that the microbial community structure in each vineyard soil has individual characteristics. Second, we assessed the fungal communities in soils obtained from eight different

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plots in one vineyard using pyrosequencing of ITS 1 in the communities. A total of 32,348 reads were obtained, and 3,181 operational taxonomic units were identified. The most abundant OTUs were assigned to *Fusarium* sp., uncultured ascomycete, and uncultured fungus. Our study comprehensively showed for the first time fungal and bacterial community structures in Japanese vineyard soils.

Population Screen of Downy Mildew (*Plasmopara viticola*) Resistant to Strobilurin-Class Fungicides in Lake Erie Regional Vineyards

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Downy mildew (*Plasmopara viticola*) remains one of the largest problems in the grape vineyard worldwide, especially in those climates that are warm and wet during the growing season. Control of downy mildew is carried out with a combination of host tolerance and chemical applications that include a range of available chemistries. Even in vineyards planted with very tolerant varieties (e.g., Concord), control is important in years with ideal pathogen conditions. Since many fungicides in use have a single mode of action, the potential for a buildup of resistance can be very high, especially when potential cultivar sensitivity might make the use of a multimode control measure untenable. This potential is well recognized in the strobilurin class of fungicides, which are important in the preemergence control of fungal pathogens in Lake Erie regional vineyards. This project has set out to ascertain the levels of strobilurin resistance in downy mildew colonies collected on diseased leaves at the end of the growing season using ARMS-SYBR Green real-time PCR to assay for the glycine to alanine mutation (G143A) known to confer a qualitative level of resistance in fungal pathogens. Preliminary data suggests that the presence of this single nucleotide polymorphism exists on high levels and warrants careful consideration by growers in their fungicide programs. Geographical distribution, population dynamics, and correlation of resistance found within vineyards composed of different grapevine genotypes were examined. This population screen is the first step to more fully understand how cultural practices affect resistance buildup in this important pathosystem.

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Interactive Effects of Mechanical Canopy Management on Pinot Grigio Grapevines

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To better understand the optimal canopy management techniques necessary to meet the demands of both vineyard and cellar, a study was conducted analyzing the interactions among canopy management steps for Pinot Grigio in the southern San Joaquin Valley. The treatments were arranged factorially where two pruning methods (spur vs. mechanical box-pruning), three shoot density levels (low [23 shoots/m], medium [33 shoots/m], and high [49 shoots/m]), and two leaf removal methods (east-side leaf removal, or none) were applied to alter the canopy microclimate in four randomized complete blocks. Pruning method and shoot density interacted to affect the count shoots and total shoots retained per meter of row. Canopy microclimate was affected by pruning method, shoot density, and leaf removal treatments. Light interception into the fruiting zone was 49% higher for spur-pruned vines compared to mechanically box-pruned vines, and was 44% higher for low shoot density compared to high shoot density treatments. A 17% decrease in leaf layer number was observed for vines with leaf removal. Yield was impacted by both dormant pruning and shoot thinning methods where an increase of 42% in mechanically box-pruned vines was seen compared to spur-pruned vines, and there was an increase of 27% from low to high shoot density. Crop load and vine vigor were impacted by the interaction of shoot density and leaf removal. Leaf area to fruit weight ratio reached the desired range (0.8 to 1.2 m^2/kg) for medium shoot density treated vines that were mechanically box-pruned. Wine phenolics analysis indicated a three-way interaction among pruning method, shoot density, and leaf removal, indicating quantitative wine parameters were multidependent on canopy management methods.

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

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Skin Anthocyanins and Phenolics Enhancement by Crop Forcing in Cabernet Sauvignon Grapes Grown in a Warm Region

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In order to significantly improve fruit quality, an experiment was conducted in 2010 to shift fruit ripening from the warm portion (July-August) to the cooler portion (September-October-November) of the growing season using crop forcing 3, 4, and 5 weeks postanthesis in Fresno, CA. Profiles of skin anthocyanins and phenolics were determined by HPLC for both crop-forced (CF) and conventionally grown (CG) Cabernet Sauvignon grapes. At harvest, CF berries had much greater total skin anthocyanins (2x) and phenolics (3x) in comparison to CG berries. Malvidin-based anthocyanins and delphidin-3-glucoside increased at a similar rate of total anthocyanins, while procyanidin and myricetin increased at a similar rate of total phenolics in CF berries. Other individual skin anthocyanins and phenolics of CF fruit increased by either a higher (5x to 6x for petunidin-3-glucoside, 3x to 4x for peonidin-3-glucoside, 5x for catechin, and 4x for coumaric acid) or lower (1x for cyanidn-3-glucoside, 2.5x for gallic acid) rate. The enhancement of malvidin-3-coumaroyl-glucoside and resveratrol decreased while petunidin-, peonidin-, and quercetin-3-glucoside increased when CF was conducted later. A small amount of petunidin-3-acetyl-glucoside and delphinidin-3-coumaroyl-glucoside was found in CF berries but absent in CG berries. Results demonstrated potential for enhancement and various sensitivities of individual skin anthocyanins and phenolics at lower temperatures when fruit ripening was shifted to the cooler portion of the growing season by crop forcing in warm regions.

Correlation of Real-Time Canopy Temperature with Soil Moisture Content and Standard Vine Physiology Measurements on Merlot and Cabernet Sauvignon Grapevines under Two Irrigation Levels

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A field trial was conducted on Merlot and Cabernet Sauvignon grapevines grafted to 1103P rootstock during the 2010 season in the California Central Valley. The goal was to correlate real-time, above-canopy temperature with soil moisture content and standard vine physiology measurements including midday leaf water potential and leaf stomatal conductance. One set of vines of each cultivar was irrigated at the standard 70% ETc level, starting 3 to 4 weeks after bloom (deficit irrigation), while another set received 90% ETc beginning at bloom (control). Watermark soil moisture sensors were installed the previous winter, and read-

ings were taken during the growing season at one-hour intervals. Shoot growth rate was monitored weekly beginning when shoots were ~3 cm long; midday leaf water potential and leaf stomatal conductance were measured biweekly starting at bloom. Real-time above-canopy temperature was recorded every 15 min with an automated monitoring system with infrared temperature sensing, starting when shoot growth rate ceased in both treatments. Shoot growth was significantly reduced by the delayed irrigation treatment in Merlot vines. Cabernet Sauvignon vines maintained their shoot growth rate even when the initiation of irrigation was delayed three to four weeks following bloom. For both cultivars, vines that had a later irrigation start date and received a smaller fraction of crop evapotranspiration (deficit irrigation) had a more negative midday leaf water potential, lower leaf stomatal conductance, and greater leaf temperature through the season compared to counterparts under a higher irrigation level and earlier irrigation start (control). Treatment differences were greater in Merlot than in Cabernet Sauvignon. Both midday leaf water potential and leaf stomatal conductance exhibited strong correlations with soil moisture content, but did not correlate with midday leaf temperature readings. Leaf temperature did not correlate with soil moisture. However, the difference between ambient and leaf temperature did correlate strongly with vine physiology measurements. This correlation improved when considering the average daily difference between air and leaf temperature versus both midday leaf water potential and midday leaf stomatal conductance. Irrigation level and cultivar also had an effect on this correlation, with the highest values in Merlot vines and the control treatment. Results show that it is possible to use leaf temperature as an indicator of vine water status, but only when related to ambient temperature.

Effect of Nitrogen Level on Root Growth of Diploid and Tetraploid Grape Rootstocks in Split-Root Technique

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Grapevines grow too vigorously to obtain high-quality berries because of wet and warm climate in western Japan. We introduced new rootstocks, which are tetraploids derived from common phylloxera-resistant rootstocks such as Riparia Gloire de Montpellier (Gloire) and Kober 5BB (5BB) induced by colchicine for controlling vine vigor and improving berry quality. These tetraploids have thicker and shorter fine roots and smaller and coarser root systems. We compared root growth response to different nitrogen levels in a split-root technique between the original diploids (Gloire and 5BB) and their tetraploids. Experimental grapevines prepared from micropropagation were grown in a growth chamber at 28°C and 16-hr day length. Grapevines were divided into two groups, and one-half of root systems of both groups was supplied with Hoagland's No. 2 nutrient solution (N 15 mM;

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solution N+) and the other half of each group was supplied with different levels of nitrogen (N 29 mM; solution N++ and 0 mM; solution N-), respectively. In all treatments, shoot growth and root growth of tetraploids of both rootstock cultivars were smaller than those of the original diploids. In N+/N treatment of both rootstock cultivars and ploidy, growth, and N contents of roots in solution N+ were higher than those in solution N-. In N+/N++ treatment, root growth in solution N++ were reduced compared with those in solution N+, especially 5BB rootstocks.

Funding support: JSPS KAKENHI 21580040.

Effect of Graft Union Height on Vine Performance and Winter Survival of Chardonnay Grapevines

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Grapevines grown in cold climates are frequently subject to winter injury and subsequent crown gall disease. Crown gall can be particularly severe at the graft union. Severe winter injury events frequently occur on clear, still nights, where the coldest air is ≈2.5 cm above the soil or snow surface. This experiment attempted to determine the possible effect of elevated graft union heights on winter injury, crown gall, and vine performance. Custom-propagated Chardonnay grapevines (clone 95) were grafted to 3309C rootstock at both standard grafting height (2.5 cm above the soil line) and at an elevated height (71 cm above the soil line, ≈10 cm below the fruiting wire). The vines were transplanted to vineyards in Hamden, CT, and Westport, MA, in 2007 and trained to a standard low wire, vertical shoot-positioned system. Immature fruit was removed from both treatments in 2008 at 650 growing degree days (base 10°C), and potential crop was estimated from fruit as being twice the weight of the immature fruit at the level of the fruiting wire. Estimated yield in 2008 for high-grafted vines was approximately twice that of low-grafted vines. There were few differences in vegetative or fruit quality parameters between treatments in 2009 and 2010. No winter injury or crown gall has been observed with either treatment. There may be benefits of high graft unions over those of traditional height regarding unmeasured cultural parameters. Except for the potential to harvest a crop in the year after planting, results to date do not suggest that high-graft unions are effective in increasing vine performance or fruit quality.

Funding support: Viticulture Consortium East.

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Economic Evaluation of Winegrape Contracts

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Winegrape sales contracts are instrumental in product procurement and risk sharing for both the winery and grower. The general objective of this study was to explore current winegrape contracting methods. Specific objectives were to: (1) characterize possible contracting methods including contracting for tonnage, acreage, and residual claimancy (such as bottle price), (2) evaluate the distribution of risk between parties for each contracting method, and (3) develop prescriptive scenarios in which each contracting method is preferred. Possible contract types considered were between a grapegrower and a winery when a grower allocates efforts among multiple activities to generate a given quality product. Comparative statics were developed using multitasking principal-agency theory. The outcome-based sharing model (i.e., "bottle price" or a grape price index) allows for asymmetric quality contributions and multitasking by both parties, input conditioning through monitoring, and double-sided moral hazard. The results show that, all else being equal, contracts offering a fixed payment per-ton likely result in lower quality fruit on average compared to compensation through a fixed base payment per-ton plus quality price incentives (i.e., a bonus/penalty for Brix, pH, TA). Regarding flexibility, the sharing contract is optimal under a wider range of circumstances than the per-acre contract. Furthermore, if any particular grape quality parameter is informative and worth measuring, it is worth incorporating into the contract by means of a bonus/penalty for that particular attribute or by other means of residual claimancy. When parameters are more costly than informative, our results recommend that the related viticultural practices should be contractually controlled by the winery. The addition of quality price incentives to a given contract when compared to a fixed payment per-ton results in one or both parties being made better off while both are at least as well off as they were without incentives.

Soil N, P, and K Affect Volatile Composition of Pinot noir Grape

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It is assumed that soil nutrient could play a very important role on grape quality; however, little is known about the actual effect of soil nutrient on grape volatile composition. This study investigated the impact of soil nutrients on free and bound volatile composition of Pinot noir grapes. Pinot noir vines grown in a sand pot were supplied with a half-strength Hoagland's nutrient solution as control or solution at various reduced nutrient levels (50% N, 20% N, 10% N, 10% P, 10% K, with 100% all others, respectively) for three consecutive growing seasoning

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(2006 to 2008). Full ripe grapes were harvested in 2007 and 2008 and the free and bound volatiles in the grapes were analyzed. Results showed that soil nutrients had great impacts on grape volatile and volatile potential compositions, and the influence in 2008 was stronger than 2007. Grapes fertilized with reduced N levels had the highest impact on terpene alcohols and C_{13}-norisoprenoids. Total terpene alcohols in 20% N and 10% N treated grapes increased ~100%, while total norisoprenoids increased ~50%, compared with the control. Free linalool and geraniol in 20% N and 10% N grapes increased 70% and 100 to 150%; the glycoside forms of linalool and geraniol increased 100% and 150%; while the bound form of vitispirane, hydroxydihydroedulan, and 3-hydroxy-β-damascenone increased 80 to 120%, 40%, and 30%, respectively. Grapes fertilized with reduced P and K levels also had increased trends for these compounds; however, the increase was much smaller than the reduced N treatment.

Influence of Regulated Deficit Irrigation and Partial Root-Zone Drying on Monastrell Grapes and Wines

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This work was undertaken to compare the effects of partial root-zone drying (PRD) and regulated deficit irrigation (RDI), applying the same amount of water, on berry composition and wine quality attributes in Monastrell grapevines under semiarid conditions. Five irrigation treatments were applied. The control treatment irrigated at 60% of the ETc (crop evapotranspiration) throughout the season (308 mm $year^{-1}$). RDI treatments were applied under conventional drip irrigation (RDI-1 and RDI-2) and under PRD (PRD-1 and PRD-2) in two consecutive years (2006 and 2007). PRD-1 and RDI-1 applied the same annual water amount (192 mm $year^{-1}$) and provided deficit irrigation from fruit set to harvest (irrigated 30% ETc) and postharvest (45% ETc). PRD-2 and RDI-2 applied the same annual water amount (143 mm $year^{-1}$) and provided deficit irrigation from fruit set to harvest (irrigated 15% ETc) and postharvest (45% ETc). An improvement in cluster microclimate positively influenced grape and wine quality in RDI and PRD. PRD-2 significantly changed flavonol and anthocyanin composition and the proportion of acylated anthocyanins compared to RDI-2. Furthermore, PRD-2 had higher total flavonol and anthocyanin content in berries than the rest of treatments. There was a strong effect of PRD on tannins in grapes and wines. The increase in anthocyanin and flavonol concentration in PRD grapes and wines (mainly in PRD-2) was due to increased flavonoids in berry skin and changes in the structure of colored

wine pigments (increasing copigmentation and polymerization processes). These changes in anthocyanin and flavonol composition under PRD may reflect a unique response to within-vine signalling induced specifically by PRD and their interaction with severe vine water stress.

Epidemiology of the Grapevine Leafroll Disease in Washington Vineyards

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The objectives of this research were to map the spatial pattern of grapevine leafroll disease (GLRD) within individual vineyards and to monitor young vineyard blocks for infection with grapevine leafroll disease. In the first objective, we monitored 25 geographically widely separated vineyard blocks for the presence of GLRD. The position of individual vines showing GLRD symptoms was recorded and plotted in a XY matrix using the row number and vine position as coordinates. Spatial maps were prepared showing the distribution of the disease in different winegrape cultivars. The results showed clustering of GLRD-infected vines along a given row in individual vineyard blocks. In some rows, two or three GLRD-infected vines were present directly adjacent to each other. In some rows, GLRD-infected vines were alternating with apparently healthy vines. These patterns of spatial distribution were observed in two different cultivars, indicating that the spatio-temporal spread of GLRD could be similar in different cultivars. This information is helping us to understand the spread of GLRD in Washington vineyards. In the second objective, two vineyard blocks planted with virus-free Syrah and Cabernet Sauvignon cultivars were monitored in 2003 and 2007, respectively, adjacent to a 20-year-old Cabernet Sauvignon block heavily infested with GLRD. In the young Cabernet Sauvignon block, a total of 17 vines showed GLRD symptoms. These symptomatic vines were located in the first four rows proximal to the GLRD-affected old block. In the Syrah block, a total of 84 vines showed GLRD symptoms and these symptomatic vines were located in rows that are proximal to GLRD-infested old blocks. These results indicated the spread of GLRD to newly planted blocks from neighboring old blocks heavily infested with GLRD. These results indicate that there is a high level of risk of disease spread to 'clean' vineyard blocks when planted in close proximity to GLRD-infested blocks. Management tactics should be applied to prevent such a spread.

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Defining Efficient Deficit Irrigation for Winegrape Cultivars Merlot and Cabernet Sauvignon under Semiarid Conditions

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Efficient deficit irrigation balances enhanced berry attributes with acceptable reduction in yield. The present study relates pre- and postveraison midday leaf water potential with the growth and composition of east and west-facing berries. Two deficit irrigation treatments were applied over four growing seasons to vines planted in north-to-south oriented rows under warm, semiarid conditions. Vines under standard irrigation were supplied with 70% of their estimated water demand. Vines under regulated deficit irrigation were supplied 50% of the standard amount between fruit set and veraison and then the same amount as standard from veraison until harvest. Midday leaf water potential and the diameter of east and west-facing berries were measured weekly. Berry maturity and phenolic composition were measured at harvest. The difference in leaf water potential between irrigation treatments pre- and postveraison for Cabernet Sauvignon averaged 0.3 MPa and the standard treatment averaged -1.0 MPa. Yield in Cabernet Sauvignon under regulated deficit irrigation was reduced 30%, berry fresh weight by 19%, and titratable acidity by 16%. The difference in leaf water potential between irrigation treatments for Merlot averaged 0.2 MPa preveraison and 0.3 MPa postveraison and the standard treatment averaged -1.2 MPa. Merlot yield under regulated deficit irrigation was reduced 25%, berry fresh weight by 11%, and titratable acidity by 9%. In both cultivars, east-facing berries under standard irrigation grew faster than west-facing berries under regulated deficit irrigation. Regulated deficit irrigation increased anthocyanin concentration per gm fresh weight by 14% in Cabernet Sauvignon and 17% in Merlot; however, content on a per berry basis was higher only in Merlot. West-facing berries of both cultivars contained less anthocyanin per berry than east-facing berries and exposure aspect differences were greatest under regulated deficit irrigation. The most efficient irrigation treatment for Cabernet Sauvignon was the standard and for Merlot was the regulated deficit irrigation.

Funding support: USDA-ARS project: 5358-21000-041-00.

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An Investigation of the Fate of *Lobesia botrana* (Lepidoptera: Tortricidae) in Infested Clusters Processed for Winemaking

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In September 2009, the first report of *Lobesia botrana* in North America was confirmed in Napa County, CA. *Lobesia botrana* larvae feed on grape berries in mid-summer and again in late summer, and infested fruit may be harvested and delivered to wineries. In 2010, wineries that processed grapes from within a quarantine area were required to press fruit to a minimum of 2 bars or unfermented pomace had to be composted, hauled to a landfill or returned to the originating vineyard. We evaluated the survival of larvae in infested Chardonnay clusters processed in a 200 pound capacity Willmes and a 3 ton Bucher press. Individual clusters, each infested with a single larva were sewn into mesh bags which constituted one-third of the clusters in each of two separate Willmes press loads. Pressure was ramped up in increments of approximately 0.25 and 0.3 bars to reach 1.5 and 1.8 bar end points, respectively. The total time under pressure for each load was 56 min. Each cluster was treated as a single replicate of a completely randomized design and data analyzed by cross-tabulation. There was no significant difference in mortality between 1.5 and 1.8 bar press loads. A single larva survived the 1.5 bar press. A surrogate pest (*Argyrotaenia franciscana*; orange tortrix) was used in 96 bags pressed in the Bucher which was taken to 1.8 bars. Two larvae survived the press run. In a destemming trial, 19% of larvae survived in berries destined for winemaking; none in the waste.

Influence of Foliar Particle Film under Deficit Irrigation on Free and Bound Volatiles of cv. Merlot (*Vitis vinifera* L.) Grapes

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Controlled water deficit is an effective way to control canopy size and modify canopy microclimate. Smaller canopy size may lead to undesirable heat stress and sun overexposure of fruit. This study investigated the effect of a kaolin-based, foliar reflectant particle film under deficit irrigation on grape-derived volatile composition. The own-rooted Merlot vines were grown in a semiarid region of southwestern Idaho. Vines were subjected to 35, 70, or 100% of estimated crop evapotranspiration (ETc) until harvest, or 35% ETc until veraison followed by 70% ETc until harvest, and particle film was applied as a split-plot within irrigation treatment with unsprayed as control. Free and bound flavor precursors were

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analyzed using stir bar sorptive extraction–gas chromatography-mass spectrometry (SBSE-GC-MS) over two growing seasons (2007 and 2008). Results demonstrated that despite vintage differences in volatile composition, deficit irrigation during berry development affected grape volatile composition. Free C_6 compounds, which give herbaceous aroma, were decreased under water stress. Deficit irrigation only slightly affected the free and glycosidically bound terpenols. However, deficit irrigation significantly increased free and bound damascenone. In addition, water stress significantly increased the concentrations of bound C_{13}-norisoprenoids including 3-hydroxy-β-damascenone, TDN, and vitispirane. Particle film application had no impact on most of the compounds, and volatile concentrations were more sensitive to water deficit status than to particle film application.

Funding support: USDA-CSREE-Northwest Center for Small Fruits Research and China Scholarship Council (state scholarship fund 2009630125).

Exposure to Sunlight Alters Fruit Composition of Norton Grapevines

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The influence of row orientation, canopy side, and cluster exposure to light on fruit composition of the *Vitis aestivalis* cultivar Norton was evaluated during the 2009 and 2010 seasons. This experiment was conducted in a commercial vineyard trained to a high bilateral cordon system. Bearing units consisted of short downward-positioned canes. Cluster exposure treatments were imposed postbloom on the shade and sun side of north-south and east-west oriented rows. The cluster exposure treatments consisted of fully exposed, partially exposed, and fully shaded. Leaf removal and/or shoot positioning was performed every two weeks to ensure proper exposure levels were maintained throughout the growing season. Individual berry temperature and photosynthetically active radiation (PAR) data were collected on two dates each season. Berries for temperature measurements were uniformly selected across the exterior surface of clusters and PAR readings were taken at the base of clusters. Row orientation and cluster exposure status had a greater impact on fruit composition than canopy side. Fruit from clusters on east-west oriented rows had higher soluble solids, anthocyanin, glucose, and fructose content and lower titratable acidity and malic acid content than fruit from clusters on north-south oriented rows. Fully exposed fruit exhibited higher soluble solids and lower titratable acidity, malic acid content, and juice potassium content than fully shaded fruit. Partially exposed fruit displayed responses that were often intermediate between fully exposed and fully shaded fruit. PAR was greatest for fully exposed treatments in both years and the east-west row orientation in 2009. Berry tempera-

tures were highest on fully exposed clusters. These results indicate cluster exposure and vineyard design factors that influence the proportion of clusters which are fully exposed require careful consideration in Norton grape culture.

Funding support: Missouri Specialty Crop Block Grant program and Missouri Wine and Grape Board.

Determining the Impacts of Crop Thinning on Vine Vigor and Fruit Quality in Pinot noir (Year 1)

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Managing crop levels is a common practice in cool-climate vineyard production to achieve vine balance and desired fruit quality, although it is a costly practice. With economic pressures, vineyard managers are questioning whether they can reduce production costs or increase yields without compromising quality. To help address this concern and to better understand vine balance and fruit quality, a crop thinning trial was started in 2010. Crop levels were moderately (35% of crop removed) or severely (65% removed) thinned at prebloom, fruit set, lag phase, or veraison and compared to nonthinned treatments in moderate (1.5 m x 2.1 m) and high-density (1 m x 1.5 m) Pinot noir vineyards. Results of year 1 indicate no differences in shoot or lateral development, leaf areas, leaf area index, pruning weights, dormant cane total nonstructural carbohydrates, basic ripeness parameters (Brix, pH, titratable acidity), total anthocyanins, or phenolics in treatments applied to either vineyard density. Very few differences were found in cluster size or architecture. However, prebloom thinning of vines in the moderate density block resulted in more berries per cluster. Within unthinned vines, basal and upper clusters were compared to determine ripening and cluster architecture differences. No differences were found in pH, TA, total anthocyanins or phenolics, cluster weight, berries per cluster, or berry weight. However, the upper clusters had higher Brix than basal clusters. Despite significant reductions in crop yield, there was no evidence that crop thinning advanced ripening or impacted vine vigor in highly vigorous vines. As the 2010 vintage was unusually cool and wet, these results may differ in a year with higher overall yields and warmer temperatures in which differences in crop levels would be enhanced. The study will continue for two more years to better understand these effects.

Funding support: Northwest Center for Small Fruits and Oregon Agricultural Research Foundation.

Viticulture – CONTINUED

Rejuvenating Syrah Grapevines with Training and Canopy Management in the San Joaquin Valley

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Canopy microclimate of Syrah06/SO4 was altered through application of three dormant pruning and two leaf thinning treatments arranged factorially in four randomized complete blocks to investigate how to rejuvenate vineyards with declining productivity. Vines were either pruned by hand to 22 nodes, or mechanically box-pruned to a 10 cm hedge, or cane-pruned by hand to six, 8-node canes arranged in opposing directions of the row with horizontal canopy separation, and leaves were removed on the east side of the canopy in the fruit zone, or not. Percent photosynthetic photon flux density (%PPFD) transmittance of mechanically box-pruned vines was 34% and 38% greater than spur- or cane-pruned vines, respectively. Leaf removal increased %PPFD by 47% compared to control. Canopy separation decreased cluster and berry weight, while increasing cluster number and yield per vine. Canopy separation delayed harvest by one week, while retaining lower juice pH and higher total acidity. Berry weight of spur-pruned vines was 15% greater than other pruning methods. Cluster number was 37% higher on cane-pruned vines with canopy separation compared to mechanically box-pruned vines, which were 22% higher than spur-pruned vines. Berry skin total phenolics at harvest of cane-pruned vines was 11% greater than spur-pruned vines, which was 8% greater than mechanically box-pruned vines. Pruning method and leaf removal interacted to affect the total iron reactive phenolics and tannin concentration in resultant wine. Crop load of cane-pruned vines was 43% and 32% greater than spur-pruned and mechanically box-pruned vines, respectively. The results from this study provide information for large-scale growers on how to rejuvenate vines that have declined in productivity.

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

Integrating Steps of Mechanical Canopy Management for Syrah in the San Joaquin Valley

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Canopy microclimate of Syrah06/SO4 was altered through application of two dormant pruning, three shoot thinning, and two leaf thinning treatments arranged factorially in four randomized complete blocks to investigate how best to man-

age crop load in the San Joaquin Valley. Vines were spur-pruned to 22 nodes or mechanically box-pruned to a 10 cm hedge, shoot thinned to 23 shoots/m of row (low), 32 shoots/m of row (medium), or 49 shoots/m of row (high). Leaves were removed on the east side of the canopy within a 45 cm zone of the fruit zone, or not. Mechanically box-pruned vines transmitted 38% more percent photosynthetic photon flux density when compared to spur-pruned vines. Fruit set was not affected by any treatment combination. Shoot density did not affect berry weight at harvest; however, berry weight of mechanically box-pruned vines was 11% lower than spur-pruned vines. Berry compensation occurred in spur-pruned and low and medium shoot density vines. Mechanical box-pruning, medium shoot density, and leaf removal 20 days postbloom gave adequate yield and manageable leaf area to fruit ratio. Yield of spur-pruned vines was 14% lower than mechanically box-pruned vines. Berry skin anthocyanin of medium shoot density vines was 63% and 59% lower than low shoot density and high shoot density vines, respectively. Berry skin total phenolics concentration was 23% lower than low and high shoot density vines. Crop load of mechanically box-pruned vines was 23% greater than spur-pruned vines. Crop load of high shoot density vines was 23% and 19% greater than low and medium shoot density vines. Leaf removal reduced crop load of Syrah by 9%. The results from this study provide information for large-scale growers on how to integrate key steps of mechanical canopy management of Syrah in the San Joaquin Valley.

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

Vine Balance Assessment of Mechanically Managed Syrah Canopies in the Southern San Joaquin Valley

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Canopy microclimate of Syrah06/SO4 was altered through application of two dormant pruning, three shoot thinning, and two leaf thinning treatments arranged factorially in four randomized complete blocks to mitigate crop load. Vines were spur-pruned to 22 nodes or mechanically box-pruned to a 10 cm hedge, shoot thinned to 23 shoots/m of row (low), 32 shoots/m of row (medium), or 49 shoots/m of row (high). Leaves were removed on the east side of the canopy within a 45 cm zone of the fruit zone, or not. The cluster number ($p < 0.0001$), leaf area to fruit ratio (m^2/kg) ($p < 0.0115$), shoots exposed per acre ($p < 0.0111$), and yield ($p < 0.0001$) increased linearly with the increase in crop load, while the pruning weight per vine ($p < 0.0001$), berry weight at harvest (g) ($p < 0.0222$), and cluster

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weight (g) ($p < 0.0011$) increased with the decrease in crop load. Leaf layer number of the Syrah canopies were better predictors of berry skin anthocyanins ($p < 0.0285$) and tannins ($p < 0.0166$) when compared to crop load ($p < 0.1435$ and $p < 0.3721$, respectively). The most preferable fruit at the farm gate was achieved with 65,000 shoots exposed per hectare, that were 5.0 cm apart along the cordon, with 3.2 leaf layers resulting in 20.5 tons per hectare yield when harvested at 24% total soluble solids. This study provides information for growers who aim to balance vine growth with fruit and quality through on-site measurements for mechanically managed vineyards in the southern San Joaquin.

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

Foliar Application of Abscisic Acid to Improve Fruit Quality and Cold Hardiness of Winegrapes

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The Ohio grape and wine industry has been expanding rapidly and demand for premium winegrapes has also increased. Unfortunately, several premium varieties do not ripen properly in the typical Ohio short growing season. This impacts not only the quality of the fruit and wine but also the cold acclimation process when grapevines overwinter. The goal of this study was to advance fruit ripening and cold acclimation of commercially important winegrape cultivars grown in Ohio; specifically the objectives were to determine the optimum foliar application rate of abscisic acid and to evaluate the vegetative and reproductive changes in response to exogenous abscisic acid application under greenhouse and field conditions. Our hypothesis was that exogenous abscisic acid absorbed by leaves can promote the fruit ripening and induce the dormancy of grapevines. We applied abscisic acid on *Vitis vinifera* Cabernet franc and *Vitis* spp. Chambourcin under greenhouse and field conditions in 2009 and 2010. Based on the results of a two-year study, the range of optimum rate and timing for foliar application of abscisic acid was determined and resulted in reduced rate of shoot growth, increased rate of periderm formation, leaf senescence, and dormancy as compared to the nontreated controls. All these changes led to increased bud freezing tolerance in treated grapevines. It is concluded that foliar application of abscisic acid has the potential to reduce freezing injury in cold-sensitive grape cultivars.

Funding support: Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center, the Ohio State University.

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New Approach for Grapevine Irrigation Scheduling Using Sap Flow Sensors: Relationship with Predawn Leaf Water Potential

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There are numerous methods proposed for vine irrigation control, but the reference standard certainly remains leaf water potential in its three forms: predawn leaf water potential (Ψ_{PD}), midday leaf water potential (Ψ_{L}), and midday stem water potential (Ψ_{S}). These parameters, obtained with a pressure chamber, are allowed identified solid reference thresholds, internationally validated and with universal significance. Nevertheless, the use of the pressure chamber involves a manual operation, impossible to automate and recording the data online. This is possible, however, with the use of sap flow technology, but for the moment, there are no reference thresholds for values of sap flow sensors. In order to take advantage of both types of measurements, a study was conducted during the 2010 season to establish a relationship between the values of sap flow (SF) and predawn leaf water potential (Ψ_{PD}). In a Viognier vineyard located at INRA Pech Rouge (Gruissan, France), eight plants were equipped with a sap flow sensor based on stem heat balance. Different vine water status was applied by drip irrigation. Ψ_{PD} was measured on the same plants and the evapotranspiration (ET_0) was calculated from meteorological data given by an INRA Climatik red station. The analysis of data shows a promising correlation ($R^2 = 0.7$, all conditions included) between Ψ_{PD} values (MPa) compared to the ratio between SF values (mm/day) and ET_0 of the preceding day (mm/day). These preliminary studies show that it is possible to establish a relationship between values obtained by both methodologies and thus extrapolate the abundance of knowledge and know-how available through the use of the pressure chamber to the application of sap flow technology. Further studies will aim at confirming the relevancy of this approach in different locations and on other varieties.

Wednesday & Thursday Poster Presentation Abstracts (Research Reports)

Industry

Continuous Heat Stability of White Wine: Industrial Application with Centrifugation and Ceramic Cross-Flow Filtration

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A method of continuous heat stability is evaluated using bentonite addition followed immediately by centrifugation and cross-flow filtration with ceramic membranes. In comparison to the traditional method of heat stabilization, this method offers potential advantages in faster processing speed and lower wine loss. New advances in centrifugation and the more robust nature of ceramic cross-flow membranes make such an innovation possible. The method can be coupled with existing continuous cold stability methods for a truly continuous stability regime. Data on the financial and wine sensory implications of the new method are presented.

Seaweed Extract Use in Agriculture: Grapes

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Seaweed extracts have been used as fertilizers and biostimulants in agriculture for many years. Although table grapes are one of the largest markets for seaweed extracts, strong interest in winegrapes has emerged in recent years. Despite this interest, many consultants and researchers are still unaware as to why and how growers use seaweed extracts in grape production. This presentation will underscore grower benefits from seaweed extracts and highlight differences between use in table and wine grapes. Data will be presented from internal Acadian AgriTech research and from external third-party work. A common advantage to growers of both table and wine grapes is to apply seaweed extracts to stimulate the elongation of the rachis to help create a more open bunch. A more open bunch allows for greater air movement through the cluster and can improve spray penetration, both of which can help reduce disease. The use of a WinRhizo root scanner to analyze treated rachises has shown an increase in length, surface area, and root volume. Optional applications near bloom have been shown to increase set, which may be desirable under some circumstances. In table grapes, the addition of seaweed extract to gibberellin sizing sprays is common. These sizing sprays, together with sprays at veraison, are used to increase fruit firmness. Sprays that increase berry set (more specific to winegrapes) and berry size (more so for table grapes) increased yield at harvest, making it important to maintain adequate nutrition to support increases in crop load. Other benefits related to seaweed extract applications include reductions in powdery mildew. Results show that when used at specific timings to target specific benefits seaweed extract can be a valuable tool for use in table and wine grape production.

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Influence of Ellagitannin Level in Wood on Extraction Rates and on Red Wine Organoleptic Properties

Julien Michel, Michael Jourdes, Thomas Giordanengo, Nicolas Mourey, and Pierre-Louis Teissedre*
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Red wine aging is generally performed in oak barrels. During this period the red wine in contact with oak wood extracts a wide variety of substances, especially ellagitannins. Their levels in red wine depend on the oak species, origin, and treatment during barrel manufacturing. Since the first characterization of vescalagin and castalagin in *Castanea* and *Quercus* woody species, six other ellagitannins have been isolated in oak wood, such as roburin A-E and grandinin. However, their impact on the sensory perception of red wine is still not well established. During our research, oak staves were classified in four groups according to ellagitannin levels estimated by the NIRS procedure (Oakscan). The ellagitannin level of each stave was also estimated by HPLC-UV-MS in order to correlate this level with the NIRS classification. Each group of NIRS classified staves was then assembled in barrels in which red wine was aged. During the aging, the evolution of the ellagitannins was monitored by HPLC-UV-MS each month and the sensory properties of the wines were estimated by a trained panel after 6 and 12 months of aging to estimate the impact of ellagitannin level. We observed a good correlation between the NIRS classification of the wood and the ellagitannins level estimated by HPLC-UV-MS. Similar correlation was also found between the NIRS classification and the ellagitannins concentration in red wine aged in the classified barrels. For example, the wine with the highest level of ellagitannins was the wine aged in the barrel that had the highest level of ellagitannins in wood. Moreover, woody and fruity aromas, astringency, and bitterness appeared to be influenced by the level of ellagitannins. However, the increases in astringency and bitterness were moderate since they did not negatively impact the overall ranking on these wines.

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Controlled Oxygenation of Musts

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The oxidation of white and rose wines is a common phenomenon usually occurring within 6 to 12 months postharvest. This oxidation can result in color changes (turning golden, slightly pink, or brown), taste characteristic changes (astringency and dryness), and aromatic alterations. Such changes lead to a devaluing of the final product and ultimate loss of consumers. This phenomenon mainly affects wines rich in polyphenols, whether from traditional pressing or inert pressing. In recent years, a sharp increase in this phenomenon has been recorded. Oxidation now affects wines that were originally thought to have an aging potential of 10 years but that are now suffering from rapid evolution (within 3 years) toward oxidation. The treatment of musts with oxygen rests on the utilization of enzymatic activities of the grape for the purpose of eliminating a portion of the polyphenols. The enzymes put into play (polyphenol-oxidase) provoke the oxidation of polyphenols, which precipitate in the form of brown pigments. During this process, certain formed compounds are susceptible to reacting with varietal aromas and ultimately destroy them. The technique of oxygenation of musts was much practiced during the 1970s, using massive doses of oxygen, hence the name of hyperoxygenation. These very large doses caused the systematic elimination of much of the polyphenols present in a must, but unfortunately, often took potential varietal aromas as well. The work of Vivelys showed that the introduction of reasonable and adapted doses of oxygen to a must can, on the contrary, turn out to be interesting in stabilizing the future wines vis-à-vis premature oxidations without destroying the aromatic potential of the product. This presentation will summarize different industrial applications from two California sparkling wineries.

A Study of the Relationship between Daily Rate of Vine Water Use and Fruit Composition

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The rate of vine water use is affected by climatic conditions, plant architecture, plant available water, and varietal specific drought resistance mechanisms. For a given climatic demand, as soil moisture decreases, vine water use subsequently declines. The decline in vine water use can be measured in real time with sap flow sensors installed on the plant. This information can be used to determine the timing of irrigation, when daily vine water use drops below a minimum value. It is also

useful to characterize the rate of vine water use decline according to the variety and to analyze the relationships between vine water use and fruit maturation profiles. We report results showing daily rate of vine water use measured across irrigated and nonirrigated vineyards during the 2009 and 2010 vintages in California and France. We compared the effect that vintage climatic conditions and irrigations have on vine water use profile across different varieties (isohydric and anisohydric). To better understand the consequences that different vine water use histories have on fruit composition, we analyzed the relationship between vine water use profiles before veraison and berry weight kinetics. We found a relationship between daily average vine water use measured before veraison and the value of peak berry weight. We also found that the relationship between peak berry weight and vine water use is varietal dependant. Implications for irrigation management according to vintage climatic context and varietal sensitivity to water deficit are discussed.

A New Approach to Tartrate Stabilization: Comparing and Optimizing the Use of Carboxymethyl Cellulose in Wine

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Since August 2009, the European Union has authorized the use of carboxymethyl cellulose in wine for preventing the precipitation of potassium bitartrate crystals. The goal of our research was to evaluate its efficacy and to understand the advantages and limitations in comparison to other stabilizing agents. Two carboxymethyl celluloses were compared with other authorized stabilizing agents, namely metatartaric acid, mannoproteins, and gum arabic. These compounds were tested in wines with medium to high levels of instability. Their stabilizing effect was evaluated by minicontact and refrigeration tests. Their resistance to acid hydrolysis degradation was also evaluated. The dosage effect was evaluated as well as its interactions with wine protein, color, and filterability. Carboxymethyl cellulose improves wine tartrate stability by interfering with the growth of potassium bitartrate crystals. It is less effective than metartaric acid but more effective than both gum arabic and mannoproteins. Its effectiveness increases using higher dosages, and differences were found between the two products tested. Because of its high negative charge, carboxymethyl cellulose interacts with wine proteins and color, causing haze and precipitation in wines that are not stable. It may also have a clogging effect on filters. Finally, carboxymethyl cellulose, gum arabic, and mannoproteins all showed resistance to acid hydrolysis. Carboxymethyl cellulose is effective for preventing potassium bitartrate precipitation. It can be used to stabilize moderately

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tartrate-unstable wines. In cases of high instability, its use can help to shorten the physical stabilizing treatment but it cannot replace it entirely. Compared to the other stabilizing agents, carboxymethyl cellulose offers a highly effective treatment option with long-lasting results. Because of its interaction with positively charged compounds in wine and its impact on filterability, it must be used in wines that are completely protein and color stable, and the timing of addition must be carefully considered.

Chitosane: A New Tool to Control *Brettanomyces* and Preserve Wine Aromatic Quality

Lucile Pic-Blateyron, Daniel Granes, **Gordon Specht**, Nathalie Sieczkowski,* and Aurélie Bornet
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Brettanomyces bruxellensis and the consequences of its development in wines are a continuous threat for wine quality. Several strategies (molecular SO_2 management, management of alcoholic and malolactic fermentations, lees management, and barrel sanitation) are useful in controlling *Brettanomyces* and its development in musts and wines. However, these strategies are not always sufficient. Moreover, there is not one specific approach that allows eliminating completely these undesirable yeasts. Recent studies led to the authorization of a new approach and product in the enological codex by the OIV in July 2009 The product, called chitosane, is of fungal origin and can be used as a new tool for control of *Brettanomyces*. Chitosane is a polysaccharide, derived from chitin, which is produced by a specific strain of *Aspergillus niger*, according to a patented process. Our research work focused on chitosane application under winemaking conditions in both laboratory and industrial winery experiments. The dosage, timing of application, and effect on *Saccharomyces cerevisiae* and *Oenococcus oeni* were investigated. *Brettanomyces* cells were studied using different methods, which included counting on plate, RT-PCR, and flow-cytometry. Industrial trials were conducted to validate the application and the efficiency of chitosane on naturally contaminated wines. Almost 30 comparative trials representing a volume of approximately 5500 hL of wine were treated at a dosage of 4 g/hL. In addition to the impact on *Brettanomyces*, the effect of chitosane on other parameters, including color, polyphenolic content, and sensory properties, was also examined. Results showed the efficiency of this new biotechnological tool for the elimination of *Brettanomyces,* even at high levels of populations from 10^5 to 10^6 cfu/mL. Chitosane can also be used on wine after alcoholic fermentation to avoid the development of a potential *Brettanomyces* contamination and thus to preserve wine quality through aging.

Industry – CONTINUED

Effect of Closure Type on Retail Price Sensitivity for Premium Wines

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The Cork Quality Council wished to compare consumer attitudes toward closure type as portrayed in focus groups to marketplace performance. Retail sales for the top 100 premium wine brands were tracked by Nielsen Surveys at four-week intervals for one year. Each of the 1,300 data points were compared to corresponding year-ago results for price and volume changes. Brands were sorted by their most prevalent closure type into categories of cork, synthetic, or screwcap. Analysis showed distinct differences in performance by closure type. Further analysis of the relationship between pricing and volume changes showed that brands with different closure types yielded different levels of correlation between pricing and volume growth. The findings indicate that price sensitivity may be linked to closure type in a manner consistent with consumer research.

Grapevine Leafroll and Vitivirus Diseases Seminar

Sequence Variation and Detection of New Zealand *Grapevine Leafroll-Associated Virus-3* Isolates

Kar Mun Chooi, Michael Pearson, Arnaud Blouin, **Robin MacDiarmid**, and Daniel Cohen*
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Grapevine leafroll-associated virus-3 (GLRaV-3) is the most damaging virus infecting grapes in New Zealand. GLRaV-3 is particularly serious with red grape cultivars because of uneven ripening of grapes and reduced anthocyanin production. Some vines infected with GLRaV-3 appear to be infected with either a low titre of virus or a strain of GLRaV-3 that has low immunoreactivity to the Bioreba reagents. We have also found that RNA extracts of some grapevines did not give PCR products using primers that were thought to be reliable. Most primers used to detect GLRaV-3 have been designed from the genomic sequence of the New York, NY1 isolate (GenBank AF037268) and GLRaV-3 antibodies from Bioreba were prepared against the NY1 strain. We have compared the apparent titre of GLRaV-3 in a range of cultivars and clones in a germplasm collection using either Bioreba reagents or polyclonal rabbit antibodies combined with an anti-rabbit-alkaline phosphatase conjugate for detection in a triple antibody sandwich format. The ratio of the apparent titres from these reagents can be used as a guide to indicate the presence of GLRaV-3 strains that differ in immunoreactivity. Molecular results have also shown that there is substantial genetic variability within the New Zealand GLRaV-3 population with the identification of both the NY1 and South African (GP18) isolates including two isolates that differ from NY1 by more than 20%. Strain specific primers have been designed for use in multiplex RT-PCR and quantitative real-time RT-PCR has been developed to screen field samples from around New Zealand. These protocols target several regions across the genome, which allows for the detection and quantification of the main New Zealand isolates.

Funding support: The University of Auckland, Corbans Viticulture, and The New Zealand Institute for Plant & Food Research Limited.

Vitis californica and Hybrids Are Hosts for *Grapevine Leafroll-Associated Virus-2* and *-3* and *Grapevine virus A* and *B*

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The objective of this research was to determine if native *Vitis* are alternate *Grapevine leafroll-associated virus* (GLRaV) hosts that might serve as reservoirs important in the continued spread of grapevine leafroll disease. One hundred fifty-two *Vitis*

Grapevine Leafroll and Vitivirus Diseases Seminar – CONTINUED

samples surrounding nine Napa Valley vineyards were collected and tested for GLRaV-1 to 5 and -9, *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), and *Grapevine virus D* (GVD) using both conventional RT-PCR and real-time quantitative RT-PCR (qRT-PCR) using the TaqMan probe. Twenty-four *Vitis* samples from three riparian areas not near vineyards were also included. DNA fingerprinting indicated that the *Vitis* samples consisted primarily of *Vitis californica* followed by *V. californica* × *V. vinifera* hybrids. GVA and/or GLRaV-3 were detected in 53% to 80% of the *V. californica* and *V. californica* x *V. vinifera* hybrids adjacent to three of the nine vineyards. In two riparian areas not near vineyards, three of the 21 *V. californica* samples were positive for GLRaV-2, GLRaV-3, GVA, and GVB. At the third riparian site, all three *V. californica* x *V. vinifera* samples were positive for GLRaV-2 and GVB. Phylogenetic analysis of GLRaV-2 and -3 partial coat protein gene nucleotide sequences indicated the isolates from *V. californica* and *V. californica* x *V. vinifera* hybrids were closely related to *V. vinifera* isolates. Although we cannot conclude anything about GLRaV-3 transmission between *V. vinifera* and native *Vitis*, we did identify a GLRaV-3 reservoir within a 2 km region of Napa County.

Funding support: American Viticulture Foundation and North Coast Viticulture Research Group.

Grapevine Leafroll Incidence, Vectors, and Impact in the Finger Lakes Region of New York

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Surveys of 95 hybrid and *Vitis vinifera* vineyard blocks in the Finger Lakes in 2006 revealed that 63 had at least some *Grapevine leafroll-associated virus* (GLRaV-1 and GLRaV-3) infected vines, with one-third having high levels (>20% of samples) of infection. Subsequent follow-up surveys revealed marked increased virus incidence from 2006 to 2008 in four of the 19 blocks (21%, 4 of 19) surveyed. Concurrent insect surveys revealed the presence of one mealybug species (grape mealybug *Pseudococcus maritimus*) and two species of soft scale (*Parthenolecanium corni* and *Pulvinaria innumerabilis*), generally at low population levels. A high percentage (>55%) of soft scale and grape mealybugs collected in leafroll-affected vineyards in 2007 and 2008 tested positive for either GLRaV-1 or GLRaV-3 or both. The combinations of GLRaV found in the insect vectors generally matched those detected in vineyards where they were collected, suggesting that the insects were responsible for vine-to-vine spread within a vineyard. In 2007, vineyards of *V. vinifera* cv. Cabernet franc (11 blocks), *V. vinifera* cv. Lemberger (two blocks), and *V. vinifera* cv. Cabernet Sauvignon (one block) were selected to evaluate differences in juice chemistry near harvest between fruits of leafroll-affected vines and healthy vines. Notably, Brix was 1 to 4° lower in leafroll-infected vines. In 2008, we focused our

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study on fruit maturity on *V. vinifera* cv. Chardonnay infected with GLRaV-1 (two blocks). Ten berries were randomly sampled from 10-paired vines each with or without visible leafroll symptoms. Overall Brix was 3 to 5° lower and pH values were slightly reduced (3.13 vs. 3.33), whereas titratable acid was higher (3 to 6 g) in leafroll-infected vines.

Funding support: USDA Viticulture Consortium–East, Lake Erie Regional Grape Program, Inc., and the New York Wine and Grape Foundation.

Statistical Parameters of Spatial Patterns of Spread for Leafroll Disease

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Grapevine leafroll-associated virus (GLRaV) is a growing concern in commercial vineyards around the world. The involvement of GLRaV in abnormal fruit maturation was first observed in the white Emperor form of the Emperor grape variety over 50 years ago and subsequently shown to be associated with a graft-transmissible agent. Despite evidence from elsewhere (e.g., Germany and New Zealand) that leafroll spreads within vineyards, it was only as recently as 2008 that spread was conclusively demonstrated in a California vineyard. GLRaV is vectored by mealybugs of various genera, mainly *Pseudococcus* and *Planococcus*. Mealybugs overwinter and hide in the bark and crotches of the trunk and cordons of the grapevine, making them difficult to spot and control with insecticides. They crawl and can be moved by wind, thus the dispersal is patchy and tends to generate localized foci of leafroll infection, which can be difficult to find early in large planting blocks. If found early, roguing can be used to control virus spread, while more highly infected blocks are often completely ripped out and fallowed before replanting. As part of a wider strategy to develop an integrated program of leafroll control, we have begun to analyze existing data on the spread of infection in time and space. Information generated by these analyses will be used in building a bio-economic toolkit to determine optimum disease management plans for individual vineyards and in the design of efficient sampling plans to detect early infection in vine blocks. We report consistent pattern dynamics among different studies and give the first estimates of statistical parameters of spatial pattern for leafroll disease.

Funding support: Napanook Vineyard and the American Viticulture Foundation.

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Frequency of Detection and Distribution of Clostero and Vitiviruses in Grapevines

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Grapevine leafroll is one of the most important viral diseases in grapevine and occurs wherever grapevines are grown. At least nine different *Grapevine leafroll-associated viruses* belonging to different genera within the *Closteroviridae* family have been reported. Typical leafroll symptoms include downward rolling and interveinal reddening or yellowing of leaves, reduced fruit size, sugar content, and yield. Vitiviruses (*Grapevine virus A, B,* and *D*) are associated with rugose wood diseases in grapevines. *Grapevine virus A* is associated with Kober stem grooving and Shiraz disease (a syndrome reported only in Australia and South Africa). *Grapevine virus B* typical symptoms are related to grafted vines and include swelling above the graft union and wood with pits or grooves that can only be seen after the bark is removed. The severity of symptoms varies with the rootstock or scion variety and the virus type, ranging from delayed budburst to vine decline and death. Eurofins STA has developed a panel (HealthCheck Panel A) that includes a combination of reverse transcription (RT)-PCR and ELISA for the specific detection of *Grapevine leafroll-associated virus-1, -2, -3, -4, -5, -6, -7,* and *-9*; *Grapevine virus A, Grapevine virus B, Grapevine virus D, Grapevine* Syrah *virus, Grapevine fleck virus,* and *rupestris stem pitting-associated virus* (including the Syrah strain). Studies in our lab have shown that the frequency of detection of Clostero and Vitiviruses is related to genome composition as well as the titer of virus present during the sampling season. Data will be presented on virus distribution and frequency of detection of these viruses using sampling methodologies developed in our lab.

A Survey for Grapevine Viruses in Virginia Vineyards

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Commercial vineyards in the commonwealth of Virginia were surveyed for *Grapevine leafroll disease* (GLRD). In red-berried winegrape cultivars (*Vitis vinifera*), leaves of GLRD-affected vines exhibited green veins, interveinal reddening, and downward rolling. In white-berried cultivars, leaves of infected vines showed mild yellowing and downward rolling. During 2009 and 2010 growing seasons, petiole samples were collected from more than 800 grapevines planted in 100 vineyards. Petiole extracts were tested for *Grapevine leafroll-associated virus-2* (GLRaV-2), GLRaV-3, and *Grapevine fleck virus* (GFkV) by one tube-single step RT-PCR using

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primers specific to a portion of the heat-shock protein-70 homolog (HSP70h) of GLRaV-2 and -3 and replicase gene of GFkV. Over 50% of the samples tested positive for one of the three viruses, with a notably high number of GLRaV-3 positives. The proportion of positive samples containing GLRaV-3 alone was higher than those with either GLRaV-2 or GFkV. In addition, GLRaV-2 and GFkV were found as mixed infection with GLRaV-3. The majority of the vineyards that tested positive for GLRaV-2 or GLRaV-3 were planted in the 1980s or before. The RT-PCR fragments amplified from select number of positive samples were cloned and their sequences compared with corresponding sequences available in GenBank. Results indicated the presence of genetically distinct isolates of GLRaV-2, GLRaV-3, and GFkV in Virginia vineyards.

Epidemiology of the *Grapevine Leafroll Disease* in Washington Vineyards

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The objectives of this undergraduate research project were to analyze the spatial pattern of distribution of *Grapevine leafroll disease* (GLRD) within individual vineyard blocks and to monitor vineyard blocks planted with "clean" materials for the risk of GLRD infection from external sources. In the first objective, we analyzed spatial distribution data collected previously from 25 geographically widely separated vineyard blocks. In these blocks, the position of individual vines showing GLRD symptoms was recorded and plotted in a XY matrix using the row number and vine position as coordinates. The spatial maps showed clustering of GLRD-affected vines along a given row in individual vineyard blocks planted with different cultivars. This information is helping us to understand the spread of GLRD in Washington vineyards. In the second objective, we monitored two vineyards blocks planted in 2003 and 2007 with GLRD-free Syrah and Cabernet Sauvignon cultivars, respectively. These two blocks are adjacent to vineyard blocks heavily infested with GLRD. In the Cabernet Sauvignon block, a total of 17 vines showed GLRD symptoms. In the Syrah block, a total of 84 vines showed GLRD symptoms. The spatial distribution of GLRD-affected vines in Syrah and Cabernet Sauvignon blocks indicated spread of the disease into these blocks from neighboring vineyards heavily infested with GLRD. These results indicate that clean plantings can be exposed to the risk of GLRD infection when planted in close proximity to disease-infested older blocks and that management tactics should be adapted to avoid the risk of disease spread from external sources.

Funding support: Research and Creative Project Award of the WSU College of Agricultural, Human, and Natural Resource Sciences; Wine Advisory Committee of the Washington Wine Commission; and USDA-NIFA Specialty Crop Research Initiative Award 2009-51181-06027.

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An Overview of Genetic Diversity among Grapevine Viruses in the Pacific Northwest

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The perennial nature of grapevines, lack of selection pressure due to their clonal propagation and multiple infections over the years can result in the buildup of a mixture of several viruses and their sequence variants within a single grapevine. Analyzing the spectrum of viruses and their variants from different grapevine cultivars will improve an understanding of the sanitary status of vineyards and provide information for the development of diagnostic tools and management practices that will lead to reduced spread and decreased economic impacts. Using molecular biology approaches, we have documented the presence of genetically distinct variants of *Grapevine leafroll-associated virus-2* (GLRaV-2), *Grapevine rupestris stem pitting-associated virus* (GRSPaV), and *Grapevine fanleaf virus* (GFLV) in the Pacific Northwest vineyards as a consequence of introduction and subsequent propagation and dissemination of infected grapevine cuttings. A detailed assessment of the molecular diversity of field isolates of GLRaV-2, GRSPaV, and GFLV in the region provided a foundation for studying their role in the epidemiology of grapevine leafroll, rugose wood, and fanleaf diseases, respectively, and developing strategies to prevent the spread of these diseases. From a practical point of view, the knowledge and resources generated from these studies offered improved capacity for the discrimination of virus strains that might escape antibody detection in clean plant programs. The project outputs have been incorporated in clean plant programs and science-based information was disseminated to stakeholders for increased awareness of viruses and their impacts on sustainability of the winegrape industry in the region. Further studies on genetic diversity of other grapevine viruses in the Pacific Northwest vineyards are being pursued.

Funding support: WSU College of Agricultural, Human, and Natural Resource Sciences Agricultural Research Center and Extension Team-Based Internal Competitive Grant, Wine Advisory Committee of the Washington Wine Commission, USDA-ARS Northwest Center for Small Fruits Research, USDA-NIFA Viticulture Consortium-West, and USDA-NIFA Specialty Crop Research Initiative Award 2009-51181-06027.

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Studies on Impacts of *Grapevine Leafroll Disease* on an Own-Rooted Winegrape Cultivar

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We conducted studies in an own-rooted Merlot block in eastern Washington to measure impacts of *Grapevine leafroll disease* (GLRD) on vine performance, fruit yield, berry and wine quality. For this purpose, grapevines were identified in a commercial vineyard in such a way that individual vines exhibiting typical GLRD symptoms and tested positive for *Grapevine leafroll-associated virus-3* were adjacent to healthy grapevines in a given row to minimize errors in sampling and experimental results due to variations in growing conditions. Data collected during 2010 season showed differences in leaf photosynthesis and chlorophyll fluorescence between GLRD-affected and unaffected leaves only after veraison. An analysis of macro- and micronutrients in leaf samples collected at two critical time points (midbloom and late veraison) from GLRD-affected and unaffected grapevines showed that they were generally within the normal range and indicated no significant differences. Fruit maturity indices (soluble solids and fruit acidity) and total anthocyanins measured at various stages of berry development showed significant differences between berries produced by GLRD-affected and unaffected grapevines. Cumulative fresh fruit yield per vine measured at the time of commercial harvest showed significant yield reduction in GLRD-affected grapevines. Small-lot wines made from grapes harvested from GLRD-affected Merlot grapevines showed significantly less pigments (anthocyanins, small and large polymeric pigments), tannins, and alcohol than in wine made from fruit harvested from unaffected grapevines. These results demonstrate that GLRD affects vine performance and negatively impacts fruit and wine quality in own-rooted Merlot grapevines grown under cool-climate conditions of eastern Washington State.

Funding support: WSU College of Agricultural, Human, and Natural Resource Sciences Agricultural Research Center, Wine Advisory Committee of the Washington Wine Commission, USDA-ARS Northwest Center for Small Fruits Research, and USDA-NIFA Specialty Crop Research Initiative Award 2009-51181-06027.

Next Generation Grapevine Virus Discovery and Detection

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Methods for analysis and detection of grapevine viruses are evolving. In previous decades, the detection and identification of viruses in grapevine relied on range of

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standard tools such as use of indicator host plants, electron microscopy, immunoassays, and PCR analysis. These tools have limitations in their usefulness in identifying "unknown" agents causing some of the diseases in grapevine. As a result, new virus species and strains and new virus introductions will not be detected by these standard assays, and they will continue spreading in the field. The advent of deep sequencing and bioinformatic data analysis will allow us to solve this problem and often result in the discovery of new viruses and virus strains. Tools of bioinformatic analysis use a metagenomic approach, wherein identification of viruses does not depend on their individual purification or detection characteristics. In this novel approach, we can characterize the entire genomes of pathogens present in a diseased vine. The sequencer will produce hundreds of millions of DNA and RNA base-sequences of the host DNA and other biotic agents present in a single experiment and the sequences assembled by computer replacing the practice of cloning single genes and sequencing and sequence analysis one at a time. The automated analysis separates the mixture of total sequences into host plant and pathogen genes. The plant parasites are then further sorted into fungal, bacterial, viroid, and viral categories. The genomes of viruses undetectable by standard means will be included with all the rest of the data. In using this new technique, very low titer or asymptomatic pathogen species could be detected.

Funding support: American Vineyard Foundation and Viticulture Consortium West.

Effect of Seasonal Changes on the Titre and Distribution of *Grapevine Leafroll-Associated Viruses* and Vitiviruses in Infected Grapevines

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A quantitative virus survey was performed for two years with three-month intervals to monitor the seasonal viral profile of *Grapevine leafroll-associated viruses* (GLRaV-1, -2, -3, -5 and GLRaV-2RG) and Vitiviruses (GVA, GVB) in infected grapevine (*Vitis vinifera*). A time-course experiment was performed in which 65 grapevine varieties previously tested to be infected with a wide range of these viruses were selected as the starting material to test the effect of seasonal changes on the virus titer. The samples were collected early in the growing season (May) in three-month intervals for two years. From May to November leaf petioles were collected, whereas in February dormant grapevine cuttings were collected. The samples were tested using three diagnostic techniques: RT-PCR, one-step qRT-PCR, and low-density arrays (LDA). A comparison between conventional RT-PCR and qRT-PCR/LDA showed that the latter two diagnostic techniques were very sensitive in detecting viruses previously undetectable by RT-PCR in different growing seasons of the grapevine. For all the tested GLRaVs and Vitiviruses, detection in

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the months of February using dormant cuttings and November using leaf petioles proved to be the optimal. A distribution study in which these viruses were qRT-PCR tested in five different sides within the infected grapevine cordon revealed that GLRaV-1, -2, -3, -4, -5, -7, -9, and GLRaV-2RG are unevenly distributed, while GVA and GVB are evenly distributed within the same grapevine.

Status of Grape-Infesting Mealybug Species and Associated Leafroll Viruses in Wine-Producing Regions of Oregon

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Mealybug species (Heteroptera: Pseudococcidae) were considered minor pests in vineyards in Oregon winegrape production areas. Recent understanding of the role these insects as vectors of the grape leafroll-associated virus complex led to intensive survey efforts in four grapegrowing regions across Oregon: Southern Oregon, Willamette Valley, Columbia River Gorge, and Walla Walla Valley. Intensive visual mealybug surveys were conducted monthly from April through October 2010 in eight vineyard sites located in these regions. Cluster mapping suggested that mealybugs spread from infested hotspots or neighboring vineyards. Late-season virus distribution symptoms were compared to mealybug distribution in order to better understand the epidemiology of virus transmission. Pheromone-baited traps specific to four mealybug species were deployed monthly to help identify species present in Oregon vineyards. Evidence of mealybugs was found in all four Oregon winegrape production areas. Grape mealybug was the only grape-feeding species present in Oregon vineyards. Observed phenology indicated a minimum of one generation per season. No vine mealybugs (*Planococcus ficus*) were documented on plants or in pheromone traps. Symptoms identical to those produced by grape leafroll-associated virus were identified visually at the end of the growing season. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction procedures were performed on collected leaves to identify any strains of virus present in symptomatic tissues. Leafroll virus strain 3 was confirmed in Southern Oregon and the Willamette Valley. In the Walla Walla Valley, sampled leaves tested positive in ELISA for at least one virus strain 4–9. No virus was detected in the Columbia River Gorge, despite the sampled vineyard being heavily infested with *Pseudococcus maritimus*.

Funding support: USDA Specialty Crop Research Initiative, Oregon Wine Board, California Competitive Grant Program for Research in Viticulture and Enology.

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