

# 67

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## 67th ASEV National Conference

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## TECHNICAL ABSTRACTS



AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

# 2016 National Conference Technical Abstracts

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## Wednesday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology — General Enology Session

#### Measurement of Organic and Inorganic Arsenic Species in Californian Wines Using LC-ICP-QQQ

**Courtney Tanabe**, Helene Hopfer, Kevin Kubachka, Susan Ebeler, and Jenny Nelson\*

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Arsenic (As) is an element naturally found in the environment, in both organic and inorganic forms and in numerous oxidation states or species. Natural sources like volcanic activity have increased arsenic levels in the environment over time. However, various anthropogenic activities such as mining and farming with arsenic-containing pesticides have also greatly increased concentrations in the environment. Arsenic is a known carcinogen and inorganic forms are primarily associated with long-term negative health effects, while the organic forms are less toxic. Therefore, determination of total arsenic content, as described by various regulations, does not provide a complete picture of the risks associated with arsenic intake.

For this study, concentrations of five different As species were analyzed in 40 California wines. The inorganic As forms included arsenite (AsIII) and arsenate (AsV), and the organic As forms included monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine (AsB). The selected wines included five different wine types: red, white, rosé, sparkling, and fortified. The samples had varying levels of alcohol content, which has previously made elemental analysis of wine difficult. The method for this project was developed for fast and accurate As speciation, which could be used for all wine types. Arsenic speciation was determined using ion exchange high-pressure liquid chromatography coupled to a triple quadrupole, inductively coupled plasma - mass spectrometer (HPLC-ICP-QQQ). The presentation will discuss the levels of the various arsenic species identified in the wines and how the levels compare to values reported by other studies for wine samples and various food products.

*Funding Support: University of California, Davis, Agilent Technologies, Inc.*

#### Clarifying the Fate of Acetaldehyde in Wine: Its Hidden Derivatives

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Red wines develop through oxidation and other aging mechanisms. Limited oxidative changes, such as color stabilization and “softening” of tannins, are desirable and contribute to wine quality and complexity. Investigations into the effects of microoxygenation on wine composition have revealed continuous oxygen consumption, yet no specific substances have proved to be useful markers for monitoring wine development. Even acetaldehyde, perhaps the major product of wine oxidation, does not appear to accumulate in microoxygenated wines. Its presence is transient because it rapidly undergoes reaction with various wine components. We believe these reactions lead to sinks into which acetaldehyde flows, some of which are hidden from common analytical methods. To overcome these shortcomings, we investigated the fate of acetaldehyde with Nuclear Magnetic

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**Enology — General Enology Session — CONTINUED**

Resonance Spectroscopy (NMR), a direct method that does not alter the sample prior to analysis and detects any substance in equal measure. Thus, it is an integral instrument to detect any form or product of acetaldehyde. One- and two-dimensional NMR was applied to analyze relevant acetaldehyde reactions with known nucleophiles (sulfites, thiols, alcohols, and flavonoids) in model wine solutions to establish basic properties of each reaction. In addition, free acetaldehyde and some of its derivatives were measured in microoxygenated wines to make sense of the inexplicable results obtained through routine analysis methods (Aeration-oxidation, HPLC-MS). Consequently, NMR measurements have begun to reveal the fate of acetaldehyde in wine: the outcome of oxidation. With this information, we hope to provide a means to predict the distribution of acetaldehyde created by oxidation into its various forms, based on a wine's composition. Given a more accurate picture of the anticipated content and the various forms of acetaldehyde, we can begin to make more predictive decisions on oxygen treatments.

*Funding Support: American Viticulture Foundation and UC Davis Viticulture Department Scholarships*

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**Cellulose Content of Bourbon Whiskey Barrels Changes over Time**

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Bourbon whiskey gains its flavor from the interaction between a distillate of a distinct mash bill and the degree to which it is aged in a new, charred white oak barrel. Reactions between the distillate and charred oak occur slowly and whiskeys are typically aged between two and 23 years. A major cell wall carbohydrate in oak wood is cellulose, a B-1,4 linked chain of glucose residues that creates a foundation for cell ultrastructure. It is unclear whether cellulose degrades in situ during aging and to what degree. To explore this, bourbon barrels of different ages were sectioned and randomly sampled to obtain averages from the numerous staves that contribute to a barrel. Using a suite of histochemical and analytical techniques, we examined whether cellulose was being degraded or left inert in the charred interior of the barrel during aging. Histochemical analysis using confocal microscopy suggested that cellulose remained intact upon charring in a new oak barrel. However, after distillate entered the barrel, age-related degradation of cellulose was clear. By measuring acid-insoluble glucose (cellulosic fraction), we confirmed that cellulose was being hydrolyzed. We quantify the progression of cellulose degradation by age. Loss of cellulose in the charred fraction into the spirit could explain some of bourbon whiskey flavor and mouthfeel, with an estimated 3 to 5 lb sugar units extracted from cellulose across the barrel surface area over six years.

*Funding Support: NSF EPSCoR*

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# Wednesday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

## Enology — General Enology Session — CONTINUED

### Impact of Wine pH Reduction by Cation Exchange on Sensory and Chemical Attributes

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Wines with both a high pH and high titratable acidity (TA) are problematic due to their increased likelihood of fault development. Most interventions to reduce either pH or TA would be exacerbated by the other parameter. Cation exchange is an effective treatment to reduce wine pH while minimally increasing TA; however, this practice is used infrequently due to the perceived complexity and concern that it will diminish wine quality. Valvin Muscat, Norton, and Chambourcin wines were treated with a streamlined cation-exchange process in both 2013 and 2014 and Syrah in 2014 only. Wines were also treated with depleted cation-exchange resin and with tartaric acid addition. Treated wines were then evaluated by descriptive analysis and chemically characterized including measurement of TA, pH, total phenolics, tannins, anthocyanins, organic acids, elemental content, ethanol, and quantification of key aroma compounds. As previously demonstrated, cation exchange reduced wine pH by exchanging  $K^+$ ,  $Ca^{+2}$ , and  $Na^+$  cations with  $H^+$ . While descriptive analysis revealed no consistent differences, results within varietal and vintage cation-exchange treatments showed reduced “yeasty” and “rotten egg” aromas in 2014 Norton and increased “cherry” aromas in 2014 Syrah. Quantification of aroma compounds by GC-MS indicated numerous, though inconsistent, changes following cation-exchange treatment. In 2013 Valvin Muscat, linalool was reduced from a mean of 1.7 to 0.9 mg/L, but no significant change was found in 2014. Vanillin in 2014 Chambourcin was reduced from a mean of 635 to 33 ug/L following cation exchange, but similar reductions were not observed in other wines. Both charged and depleted resin treatments caused reduced ethanol between 0.2 to 0.7% ABV, with most of the ethanol removal occurring at the start of treatment, suggesting the reduction was due to wine/resin interaction rather than cation exchange.

*Funding Support: Missouri Department of Agriculture and the Missouri Wine Marketing and Research Council*

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**Enology — General Enology Session — CONTINUED**

**Combination of Cold Soak and Whole-Cluster Fermentation in Pinot noir: Effect on Color, Phenols, and Sensory Properties**

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Pinot noir grapes were made into wine by combining cold soak for five days with dry ice (CS) and with 20% (by weight) whole-cluster additions (CS + WC), a traditional practice for Pinot noir production. A control treatment consisting of 10 days skin contact and a control treatment with whole cluster addition (control + WC) were also produced, each replicated three times ( $n = 3$ ). There was neither sugar consumption during CS, nor any effect of CS on alcoholic fermentation. At pressing, anthocyanins by HPLC were more abundant in control wines, while tannins and total polymeric pigments were more abundant in the CS and CS + WC treatments, indicating that only CS positively increased tannins. Color intensity was initially higher in CS and CS + WC wines and these differences were maintained after malolactic fermentation, but subsided after one year of bottle aging. Wines were submitted to descriptive sensory analysis by a trained panel both at bottling ( $n = 12$ ) and after three months of bottle aging ( $n = 20$ ). At bottling, a correspondence analysis and Generalized Procrustes analysis indicated that CS wines had highest color intensity, aroma, and astringency scores, while CS + WC and control + WC wines had a similar (and positive) sensory impact consisting of enhanced fruit and herbal notes. After three months of bottle aging, control + WC and CS wines had greater color intensity while control + WC and CS + WC wines were perceived as having more pronounced floral and almond-like character and greater astringency and bitterness. Although WC addition to Pinot noir fermentations played little or no role on tannin and anthocyanin extraction, this practice does have a measurable positive effect on overall wine aroma and can also modulate astringency and bitterness.

*Funding Support: INTA*

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## Wednesday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Viticulture — Disease Management Session

#### Superior Salt Tolerance in Grafted Accessions of Wild *Vitis* Species

**Kevin Fort**, Claire Heinitz, and M. Andrew Walker\*

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Soil salinity can reduce yields in grape at levels as low as 2.5 dS/m ECe, a level commonly exceeded in salt-affected viticultural regions of California. Viticultural soils in these areas can have double this salt concentration and some promising but undeveloped sites have triple. Using a rapid screen for chloride exclusion, 326 accessions of wild American *Vitis* species were tested for chloride exclusion. The best-performing genotypes were subsequently assayed for rooting ability. Six genotypes were selected that exhibited both strong chloride exclusion and high rootability from dormant cuttings: three accessions of *Vitis acerifolia* and one accession each of *V. treleasei*, *V. rupestris*, and *V. girdiana*. These accessions and ten commercial rootstocks were grafted with a Cabernet Sauvignon scion and grown in large containers in a shadehouse in 2014 without salinization. In 2015, NaCl was increased gradually over one month to a final concentration of 75 mM and maintained for an additional two months. All ten commercial rootstocks and two of the six wild accessions showed some combination of high chloride concentration in the leaves, characteristic chloride-induced leaf necrosis, nutrient deficiency symptom in the leaves, or leaf loss. Four of the six wild accessions were asymptomatic, had shoot biomass comparable to the commercial rootstocks, and had relatively low chloride uptake into the leaves, even compared with the strongest chloride-excluding commercial rootstock, Ruggeri 140. The two strongest excluders, accessions of *V. acerifolia* and *V. treleasei*, had 29 and 32% of the chloride accumulation measured in Ruggeri 140, respectively. A nutrient analysis of the leaves is currently being conducted. These results support the utility of the rapid chloride screen, the existence of superior germplasm for salt tolerance, and the possibility of improved nutrient acquisition under high salinity conditions.

*Funding Support: E&J Gallo Winery, the California Grape Rootstock Improvement Commission, the California Grape Rootstock Research Foundation, the American Vineyard Foundation, the CDEA Improvement Advisory Board, the California Table Grape Commission, and the Louise Rossi Endowed Chair in Viticulture.*

#### Further Understanding the Cause and Management of Sour Rot

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Sour rot, a disease affecting grapes near harvest, has been observed in viticultural regions worldwide, yet much about basic disease biology and effective management remains unknown. It is characterized by rotting of grapes in the weeks preceding harvest, accompanied by the sour smell of acetic acid and the presence of *Drosophila* (fruit fly) species. We successfully reproduced the visual symptoms of sour rot and the characteristic accumulation of acetic acid following an initial production of ethanol by wounding healthy berries in the lab, inoculating them with *Saccharomyces cerevisiae* and *Acetobacter aceti*, and exposing them to *D. melanogaster* adults. Inoculation had a significant effect on ethanol production but

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Viticulture — Disease Management Session — CONTINUED

not on generation of acetic acid. In contrast, exposure to *Drosophila* resulted in substantial acetic acid production accompanied by declining levels of its ethanol precursor, indicating that acetic acid production is dependent upon a factor or factors introduced by the flies, one of which is likely microbiological. The role of *Drosophila* in disease development was clarified using field trials conducted on the interspecific hybrid cv. Vignoles in 2013 to 2015, in which applications of both insecticide and antimicrobial treatments significantly reduced sour rot incidence and severity. In the 2015 trial, untreated control vines averaged 20.5% sour rot severity, while those receiving a combination of both the insecticide zeta-cypermethrin (Mustang MAX) and the antimicrobial hydrogen dioxide (OxiDate 2.0) weekly starting prior to the appearance of sour rot symptoms had 4.0% severity, an 81% reduction. Those receiving only insecticide sprays saw a 49% reduction in severity. These trials further support the hypothesis that a yeast-bacteria complex, in conjunction with the presence of *Drosophila*, is responsible for the development of sour rot and indicate that targeting these organisms can provide significant control of the disease.

*Funding Support: New York Grape and Wine Foundation, New York Specialty Crop Block Grant*

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**Identifying Economic Hurdles to Early Adoption of Trunk Disease Preventative Practices in California Winegrape Vineyards**

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Trunk or wood canker diseases pose a serious threat to winegrape growers. Despite high prevalence and substantial consequences, growers routinely wait to adopt field-tested, preventative practices (delayed pruning, double pruning, or application of pruning wound protectant) until the vineyard presents symptomatic vines (~10 years old). We investigated why growers are not adopting preventative practices earlier. To do so, we quantified the gains from adopting practices at different ages in infected vineyards by simulating winegrape production in different crush districts in California using a bioeconomic model parameterized with scientific evidence on trunk diseases, preventative practices, and vineyard costs and returns. We found growers are better off adopting a preventative practice when the vineyard is infected and are best off adopting in a three-year old vineyard. The profitable lifespan of an infected vineyard can increase by >50% when practices are adopted in young vineyards. However, it takes two to 10 years for practices adopted in year three to outperform no action; 0 to eight years when adopted in year five; and 0 to four years when adopted in year 10, likely leading growers to perceive preventative practices as less effective than they are. When practice costs are <\$100 per acre and the perceived risk of infection is >1%, early adoption is preferred to waiting. When practice costs are >\$200 per acre, however, adoption occurs when the probability of infection is close to one, indicating many will wait for certainty before adopting. Lastly, at very low levels of perceived infection risk, the earliest adoption may not be optimal because gains in net returns from adopting early in

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### Viticulture — Disease Management Session – CONTINUED

an infected vineyard do not outweigh the costs if the vineyard is healthy. To alleviate delay in adoption until symptoms are present, we see a need to further inform growers of the benefits of early adoption using effective extension tools.

*Funding Support: United States Department of Agriculture*

#### Impacts of Grapevine Leafroll and Red Blotch Diseases in Washington Vineyards

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Grapevine leafroll (GLD) and red blotch (GRBD) diseases are significant threats to the productivity of Washington vineyards. Studies were conducted in commercial vineyards to examine impacts of GLD and GRBD on fruit yield and quality in own-rooted, red-fruited winegrape cultivars. In each cultivar, 15 to 20 symptomatic grapevines and an equal number of asymptomatic vines were selected for this study. Vines showing symptoms of GLD and GRBD and those without symptoms were tested by molecular diagnostic assays to ensure that symptomatic vines were positive for *Grapevine leafroll-associated virus 3* (GLRaV-3) or *Grapevine red blotch-associated virus* (GRBaV) and healthy vines were negative for the two viruses. Data on fruit yield (number of clusters/vine and total weight/vine) were collected at commercial harvest in September/October of 2015, depending on the cultivar. At commercial harvest, ~30 berries/vine were collected randomly from different clusters of symptomatic and asymptomatic grapevines and fruit quality was assessed. The data were analyzed by two-tailed Student's *t*-test to determine significant differences. Symptoms of GLD and GRBD were consistently observed after veraison in all cultivars studied, even though GLRaV-3 and GRBaV can be detected throughout the season. No differences in leaf physiological parameters between healthy and asymptomatic leaves of virus-infected vines were observed preveraison. In contrast, appreciable differences in leaf physiological parameters between healthy and symptomatic leaves of vines infected with GRBD were observed postveraison. The results indicated that GLD and GRBD mainly affect sugar accumulation in berries and that in some cultivars, disease can significantly reduce fruit yield. However, the observed negative impacts were variable among winegrape cultivars and seasons and in different geographic locations. These results provided some insights on how two distinct viral diseases affect vine health and fruit quality in Washington vineyards.

*Funding Support: WSU-Agricultural Research Center, WSDA-Speciality Crop Block Grant Program, Washington Wine Commission, and Washington State Grape and Wine Research Program*

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Viticulture — Disease Management Session — CONTINUED

**Molecular Strategies to Stack Powdery Mildew Resistance from Multiple Backgrounds in a Grape Breeding Program**

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The stacking of multiple resistance genes with different mechanisms is a proven breeding strategy to improve the durability and the level of resistance. This strategy requires identification of multiple sources of resistance, functional characterization of resistance mechanisms to prioritize optimal genetic combinations, and genetic markers that allow the different resistances to be monitored through crosses among themselves and with elite cultivars. This study used and developed genetic markers to eight grape powdery mildew resistance sources: *Muscadinia rotundifolia*, the Chinese species *Vitis piasezkii* and *V. romanetii*, and an array of *V. vinifera* cultivars and *V. sylvestris* accessions from central Asia. Multiple steps are required to combine resistance from two or more genetic backgrounds while maintaining high fruit quality attributes and other important horticultural traits. First, a resistance locus is introgressed into a high-quality variety. Superior individuals with the resistance gene are then selected and selfed to develop homozygous resistant lines for each resistance source. Progeny are selected from these homozygous resistant lines with the goal of combining multiple resistance forms via marker-assisted breeding. At all stages of the breeding program, seedlings and plants are tested for genetic markers and powdery mildew resistance is confirmed by careful evaluation for disease development in field, greenhouse, and *in vitro* assays. The stacking of resistance from three sources into one line and the development of a fourth source for later introgression are presented.

*Funding Support: We gratefully acknowledge support for Daniel Pap from Corvinus University of Budapest, Hungary, and research funds from the Louise Rossi Endowed Chair and the Louis P. Martini Endowed Chair*

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## Wednesday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Viticulture — General Viticulture Session

#### Comparison of Nitrogen Management Schemes in Cover-Cropped Vineyards

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Vineyards in the eastern United States are often characterized by excessive vegetative growth. Cover cropping is a common strategy to reduce vine vigor. Cover crops provide a myriad of agronomic benefits, but can compete for water and nutrients. Many cover-cropped eastern vineyards experience low vine tissue nitrogen and depressed berry yeast assimilable nitrogen (YAN). Low vine tissue and juice nitrogen can have negative impacts upon fruit yield and wine aroma, respectively. Previous research indicated that soil-applied nitrogen was effective at improving vine capacity and tissue nitrogen, but foliar urea was most effective at improving berry YAN. Foliar- and soil-applied nitrogen treatments were imposed upon three commercially managed sites planted to Petit Manseng, Sauvignon blanc (*Vitis vinifera*), and Vidal blanc (*Vitis spp.*). Treatments were imposed at the Sauvignon blanc site for five years. The Petit Manseng and Vidal blanc were subjected to treatments for two years. Petiole nitrogen and leaf chlorophyll content were significantly increased by soil nitrogen amendments. Soil-applied nitrogen was more effective at increasing crop yield and pruning weights than was foliar-applied nitrogen. Yields improved in the long-term experiment after four years of application. In the Sauvignon blanc vineyard, fruit:pruning weight ratios decreased with increasing rates of soil-applied nitrogen. Enhanced point quadrat analysis indicated that nitrogen treatments had no effect upon canopy architecture. Foliar urea sprays were very effective at increasing juice YAN and did not significantly alter the ammonia:primary amino nitrogen ratio. Foliar urea significantly increased most individual amino acids measured. However, foliar urea treatments were least effective at increasing individual amino acids in Vidal blanc. A nitrogen management strategy comprising both soil and foliar applied nitrogen may be the most effective means to maintain vine and must nitrogen status, while retaining the benefits of a cover crop.

*Funding Support: National Institute of Food and Agriculture, US Department of Agriculture, under agreement No. 2010-51181-21599*

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Viticulture — General Viticulture Session — CONTINUED

**Grapevine Root Cold Hardiness**

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Grapevine buds have a distinctive cold acclimation and deacclimation pattern. Whether these patterns also occur in roots is unknown. While direct yield loss is distinct when buds are damaged, grapevine root damage can be equally devastating: delayed or uneven budbreak, weak shoot growth, and canopy collapse. If root cold hardiness thresholds and/or acclimation patterns were better understood, mitigation strategies could be developed to reduce the likelihood of root damage. This study had two major objectives: (1) to define whether roots have the ability to acclimate and (2) to elucidate their maximum cold hardiness. Due to the lack of clear protocols for assessing grapevine root cold hardiness, simultaneous protocol optimization and cold hardiness evaluations were done on potted *Vitis vinifera* Merlot and Chardonnay grapevines. To determine whether grapevine roots acclimated in response to their environment, three different preconditioning regimens were used: ambient air temperature when vines were actively growing, 12°C for a minimum of one week during dormancy, and 0°C for a minimum of one week during dormancy. After each preconditioning regime, root samples were taken and subsequently exposed to programmed temperature regimes of either -2.0, -4.0, -6.0, or -8.0°C. Electrolyte leakage, the gold standard in root cold damage studies, was used as the primary index of damage while low temperature exotherms (collected through differential thermal analysis) were measured as a potential alternative for assessing damage. Overall, Chardonnay and Merlot root systems did not have drastic acclimation patterns: little variation in absolute cold hardiness was seen regardless of preconditioning (<1.2°C). Maximum root cold hardiness was experimentally derived for both varieties (Chardonnay median -5.9°C, Merlot median -5.7°C). Additionally, we found differential thermal analysis an effective means to determine the temperature at which grapevine roots are damaged by cold.

*Funding Support: Washington State University Department of Horticulture, Washington State University Viticulture and Enology Program, Washington State University Irrigated Agriculture Research and Extension Station*

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## Viticulture — General Viticulture Session — CONTINUED

### Influence of Berry Development on the Activity of Tannin in Partial Extracts

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Tannins derived from grapes are extracted during wine production operations and are responsible for red wine astringency. Tannin structure changes throughout berry development, but it is unclear to what extent the corresponding tannin activity changes. Furthermore, knowledge of the role of berry development with regard to the corresponding tannin extractability remains incomplete. The purpose of this investigation was to determine the role of berry development on tannin extractability and activity. The development of grape berries (*Vitis vinifera* L. cv. Cabernet Sauvignon) in ~70 commercial vineyard blocks in Napa Valley was monitored from veraison through commercial harvest. The skin and seed of sampled berries were extracted separately and exhaustively in 2:1 (v:v) acetone:water and compared to a subsample of berries that were crushed and then partially extracted in a model wine system. Extracted tannins were analyzed to determine concentration, molecular size distribution, subunit composition, and pigmented tannin, with this information being compared to corresponding tannin activity. Tannin activity declined through much of fruit ripening, although for most blocks there was a significant increase in the latest period of berry development. Based upon tannin subunit analysis, the decline in tannin activity was associated with a proportional reduction in seed tannin extraction. From principal component analysis, tannin activity was most closely associated with tannin molecular size and pigmented tannin contribution. Based upon climatological, geographical, and vineyard management information, factors related to tannin structure and activity will be discussed. Overall, the results of this study give new insight into tannin structure-activity relationships which dominate during fruit ripening.

*Funding Support: American Vineyard Foundation, California State University  
Agricultural Research Institute*

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Viticulture — General Viticulture Session — CONTINUED

**Effects of Undervine Weed Management Methods on Vine Physiology, Yield, and Grape and Wine Composition**

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The spraying of herbicides to keep the undervine area free from competing vegetation is a common practice in vineyards. However, as concerns about negative effects of herbicides rise and with increasing numbers of growers wanting to grow grapes in a sustainable manner, alternative methods to herbicides must be investigated. A three-year trial on the effects of various forms of vegetation management in the undervine area of vineyards in New Zealand was therefore undertaken. Herbicide spray was compared with undervine cultivation or mowing. The trial was carried out in Pinot noir and Sauvignon blanc vineyards in the Marlborough region and in Merlot and Syrah vineyards in the Hawke's Bay region. Trials were set up as randomized block designs with six replications of each treatment in each variety. Vines with undervine mowing showed reduced vegetative growth in the first year, but devigoration only became evident in the cultivation treatment in the third year of the trial. The change in vine trunk circumference over the course of the trial showed the largest gain from the herbicide treatment and the smallest gain from the mowing treatment except in Sauvignon blanc. Harvest yield was generally lower in the mowing treatment, at least partly explained by reduced berry size. Mowing significantly and consistently reduced YAN compared with herbicide in all varieties, but there were few other differences in must basic composition. There was a consistent reduction in IBMP in Sauvignon blanc fruit and wine due to mowing. The treatments also significantly affected the species of plants found in the undervine area at the end of the study. This shows that choice of undervine weed management strategy has significant effects on vine growth and productivity, potential wine quality, and the ecology of the vineyard as a whole.

*Funding Support: New Zealand Winegrowers, MAF Sustainable Farming fund, EIT*

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# Thursday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

## Enology — Microbiology Session

### Isolation and Characterization of [*GAR*<sup>+</sup>] Yeasts in Commercial Stuck Fermented Wines

**Minami Ogawa**, Lucy Joseph, and Linda Bisson\*

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Stuck fermentations are a problem during winemaking in which sugar metabolism arrests or slows, resulting in high residual sugar and microbial instability. One factor that contributes to fermentation arrest is the inability of the yeast to adapt to its surrounding environment. This adaptive stress tolerance comes at the cost of reduced metabolic rates as *Saccharomyces cerevisiae* adjusts its metabolism to enable survival. One mechanism for altering metabolism in a heritable way is via the establishment of heritable protein states or prions. One such prion is the [*GAR*<sup>+</sup>] prion, often induced under stress conditions. Typically, *Saccharomyces* shows glucose repression in the presence of glucose. This prevents it from using other carbon sources when glucose is available. [*GAR*<sup>+</sup>] or prion-induced yeasts can use alternative carbon sources in the presence of glucose; as a consequence of loss of glucose repression, sugar consumption is reduced. The [*GAR*<sup>+</sup>] prion can be induced by the presence of bacteria.

To determine whether prion induction occurs under commercial wine fermentation conditions, 127 samples of arrested wines were obtained from 40 wineries. The yeasts from these samples were isolated and three to 16 single colonies were picked from each wine. The yeast colonies were screened for presence of [*GAR*<sup>+</sup>] on selective medium to detect the presence of the prion. Twenty-eight out of the 127 sampled wines exhibited yeast with [*GAR*<sup>+</sup>] characteristics. From 6.25 to 87.5% of the isolated colonies from these 28 wines expressed good growth on the prion-selective medium. To confirm the prion state, yeast cells were desiccated for a month to cure the [*GAR*<sup>+</sup>] state and validate that the phenotype enabling growth on the medium was due to the presence of the prion. This work demonstrates that prion induction can be observed under commercial production conditions.

*Funding Support: American Vineyard Foundation*

### Understanding Nutrient Use Efficiency in Wine Yeasts and Its Role in Problem Fermentations

**Ayca Ozcan**, Nicholas Simmons, Larry Lerno, Vladimir Jiranek, Dario Cantu, Anita Oberholster, and David Block\*

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Wine yeasts vary quite dramatically in their efficiency in converting grape juice nutrients into active biomass. Wine yeast strains that are grown in the same medium or juice will reach different maximum cell concentrations. In general, strains that reach a greater cell concentration will have fewer problems completing fermentations. We have taken a multifaceted approach to understanding the metabolic and regulatory differences among yeast strains that cause these differences in nutrient utilization efficiency (NUE). Initially, we focused on four commercial wine yeast strains with varying NUE, Montrachet, Cote des Blanc, T306, and Uvaferm 43, using small scale (400 mL) fermentations in synthetic MMM me-

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**Enology — Microbiology Session — CONTINUED**

dium. The maximum cell concentration reached by these strains ranged from an  $OD_{600\text{ nm}}$  of 5.3 to 7.9. We analyzed the intracellular and extracellular metabolites during fermentation using GC-MS and HPLC-RI, respectively. Complete analysis of the lipid profile of each strain was performed using Triple Quadrupole LC-MS. In addition, we used a transcriptomic approach (RNA-Seq) to understand relevant transcriptional control mechanisms. Using principal component analysis with initial metabolomic data, we observed that certain key metabolic pathways are most relevant in determining NUE, including the pentose-phosphate pathway, TCA cycle, and fatty acid synthesis. To further confirm our results, we used a series of wine yeast mutants that overexpress key pathway enzymes.

*Funding Support: American Vineyard Foundation, Ernest Gallo Endowed Chair in Viticulture and Enology*

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**Impact of Bacterial Load and Oxidative Stress of the Grape Must in Problematic Fermentations**

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An arrested or a sluggish fermentation is expensive for a winery in multiple ways. Careful management of nutrients, strain selection, and fermentation conditions dramatically reduce this risk. However, instances of problematic fermentations arise periodically in spite of prudent management. Our study focused on defining the causes underlying the yeast strain's poor fermentation performance in reference to identified stress factors found in the difficult-to-ferment juices. The initial microbial load of grape must is known to impact yeast fermentation. The inhibitory role of previously identified lactic acid bacteria in yeast fermentation was confirmed and these bacteria are efficient at inducing establishment of the [ $GAR^+$ ] prion in wine strains. This prion is a protein conformational change, inherited by the progeny cells, which alters the metabolic state of the yeast which has a reduced fermentation capacity. Several lactic acid and acetic acid bacteria isolated from stuck wines produced varying levels of acetate and lactate and were also found to be capable of inducing the [ $GAR^+$ ] prion in many wine yeast. Oxidative stress is another factor that can affect fermentation efficiency. Simulating oxidative stress by adding the oxidizing agent hydrogen peroxide ( $H_2O_2$ ) leads to difficulty in fermentation progression and the accumulation of mannitol by the yeast. The level of mannitol produced during fermentation peaks seven to nine days after oxidative stress exposure, though the level is juice- and strain-dependent. Samples were collected for exo-metabolome analysis of  $H_2O_2$ -treated and untreated juices using juices known to induce mannitol accumulation when oxidized to identify the components of the juice that change under these conditions. The goal of this work is to identify biomarkers in the juice that predict stress issues.

*Funding Support: American Vineyard Foundation*

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## Thursday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology — Microbiology Session — CONTINUED

#### **Influence of *Oenococcus oeni* and *Brettanomyces bruxellensis* on Hydroxycinnamic Acids and Volatile Phenols of Aged Wine**

Mikkel Gulmann Madsen, **Nathalia Kruse Edwards**, Mikael Agerlin Petersen, Lucky Mokwena, Hentie Swiegers, and Nils Arneborg\*

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The effect of two *Oenococcus oeni* strains, Viniflora CiNe and CH11, with and without cinnamoyl esterase activity, respectively, on the concentrations of the hydroxycinnamic acids (HCAs) *p*-coumaric and ferulic acid and their respective volatile phenols, 4-ethylphenol and 4-ethylguaiacol, was investigated over six months in Cabernet Sauvignon wines inoculated with two different *Brettanomyces bruxellensis* strains, CBS 73 and CBS 2499. Both CiNe and CH11 showed growth and malolactic fermentation (MLF) in the wines. There were no clear growth interactions between *B. bruxellensis* and *O. oeni*. Furthermore, *B. bruxellensis* did not inhibit MLF by CiNe or CH11. The HCA concentrations in all wines increased until 114 days of fermentation, after which they decreased from day 114 to day 180. Wines with CiNe had the highest concentrations of HCAs throughout fermentation. However, even though CiNe degraded more of the tartaric ester-bound forms of HCAs into free HCAs, there was no clear difference in the production of volatile phenols among wines with CH11 and CiNe and the two strains of *B. bruxellensis*. Thus, it seems that the concentration of volatile phenols in wine depends more on strain differences of *B. bruxellensis* than on cinnamoyl esterase activity of *O. oeni*.

*Funding Support: Chr. Hansen A/S, University of Copenhagen*

#### **Identifying Native and Immigrant *Saccharomyces* Yeast Strains**

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Using spontaneous fermentations for wine production is increasingly popular with many wineries and wine consumers. However, it is unknown whether such fermentations are being carried out by an autochthonous (indigenous) strain of *Saccharomyces*, by a commercial strain, or by an autochthonous-commercial hybrid. To help answer this question, we collected yeast primarily from hardwood trees in wine regions of California that are remote from commercial yeast sources. The sites were at least five kilometers from wineries, breweries, or bakeries. Samples were enriched in grape juice medium and organisms responsible for fermentation were selected and identified using 26S rDNA sequence analysis. A variety of different yeast species were isolated including twelve *Saccharomyces cerevisiae* and one possible *Saccharomyces paradoxus*. Other isolates included seven *Lachancea thermotolerans*, five *Zygosaccharomyces pseudobailii*, two *Pichia galeiformis*, one *Torulasporea delbrueckii*, one *Cryptococcus diffluens*, one *Rhodospiridium diobovatum*, four *Metschnikowia*, and three *Candida* species. Microsatellite DNA analysis was used to compare our isolates to commercial strains and determine whether there is a pattern characteristic of *Saccharomyces* that are native to western North America. The microsatellite system used 10 primer sets in 10 genes over eight chromosomes

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Enology — Microbiology Session — CONTINUED

labeled with three fluorescent dyes. An extensive database of allele fragments and sizes is available online for this set of primers. This data set allowed us to compare our isolates to strains from other regions of the world and to commercial strains. We could determine whether strains in the culture collection and from spontaneous fermentations are related to commercial strains or are autochthonous. Commercial and North American *Saccharomyces* strains were distinct by cluster analysis. This ability could help us define California microbial terroir and could potentially help winemakers produce wines with more distinct regional character.

*Funding Support:* UC Davis Department of Viticulture and Enology

**Impact of Yeast Flocculation and Biofilm Formation on Yeast-fungus Co-adhesion in a Novel Immobilization System**

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A novel method of yeast immobilization, called biocapsules, has been developed in which yeasts are attached to the hyphae body of the fungus *Penicillium chrysogenum* (Peinado et al., 2004). Yeast immobilization allows higher cell densities than traditional fermentation methods, yield improvement, reuse of the biocatalyst, etc. Biocapsule immobilization has been used to produce white, sweet, and sparkling wines and in bioethanol production (Peinado et al., 2005; Puig-Pujol et al., 2013; García-Martínez et al., 2015; García-Martínez et al., 2011). In a yeast screening for fungus co-immobilization, flor strains formed the most consistent biocapsules. These yeast naturally form a biofilm on the liquid surface of sherry wines, in which the cell wall Flo11 protein plays an essential role. Loss of *FLO11* in a flor strain decreased immobilization five-fold, indicating that flor formation likely influences yeast-fungus co-immobilization. Biofilms form on interphases (solid or liquid) via cell-cell and/or cell-surface attachment. Flocculation occurs in liquid media and refers to the attachment of cells to each other when grown dispersed. Flo11 protein is essential in biofilm formation and also participates in flocculation. To elucidate the influence of biofilm formation versus flocculation on yeast-fungus co-immobilization, 19 biofilm-forming and flocculating yeast strains were identified from a screen in the UCD VEN collection and their ability to flocculate and form biofilm was quantified. Strains displaying different abilities to flocculate or form biofilms were also evaluated for their ability to immobilize with *P. chrysogenum*. Nine strains showed different patterns of flocculation/biofilm formation, making them interesting candidates for immobilization. These results illuminate parameters that influence yeast-fungus co-immobilization that might lead to improvement of biocapsule consistency and further extend the field of application for this new immobilization system.

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## Thursday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology — Phenolics Session

#### Impact of Cabernet Sauvignon Berry Maturity on Wine Anthocyanin, Tannin, and Polymeric Pigment Content over Time

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Maturity of red grape berries is an important factor in the phenolic composition of both the fruit and the corresponding wines. Fruit maturity impacts the concentrations of tannins and anthocyanins and the ratio of anthocyanins to tannins (A:T) found in the corresponding wines. In this study, Cabernet Sauvignon grapes were harvested at soluble solids concentrations of 20, 24, and 28 Brix and made into wine (200L scale) to produce wines with different A:T ratios. Wine samples were collected in triplicate at day 10 of fermentation. The phenolic fraction was then isolated and dissolved in model wine. The model wine samples were incubated at elevated temperature (30°C) for four months and sampled to evaluate changes in anthocyanins, tannins, and polymeric pigments. Initial A:T ratios were 0.43, 0.98, and 0.68 for the 20, 24, and 28 Brix treatments, respectively. Results of two-way ANOVAs indicated that winemaking treatment and time significantly affect polymeric pigment formation and anthocyanin decline, but tannins were only affected by treatment. Anthocyanins declined significantly in all treatments (-50% decline from time 0 to one month) and a time x treatment interaction was observed for small polymeric pigment (SPP) formation, indicating that treatment impacted their formation. The 20 and 24 Brix treatments had increases in SPP throughout, while in the 28 Brix treatment, SPP only increased during the first month. Time and treatment effects were seen for the large polymeric pigment formation, which increased significantly (12.6-fold in two months). Results show picking decision alters polymeric pigment formation. The 24 and 28 Brix treatments formed similar and the greatest amount of polymeric pigments, suggesting that fruit ratios of A:T closer to 1.0 favor polymeric pigment formation in Cabernet Sauvignon wines.

*Funding Support: Washington Wine Grape Funds*

#### The Influence of Antioxidants and Microbes on Outcomes of Microoxygenation Treatments in Red Wines

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Microoxygenation is a widespread technique in red winemaking, but no chemical measures can be used to predict the outcome. Specifically, there is no reliable way to determine prior to treatment how a given wine will react with oxygen consumption and acetaldehyde production. The presence of antioxidants such as sulfur dioxide and the presence of yeast and bacteria seem to modulate these processes, so a series of experiments were conducted to clarify the roles that these variables play in microoxygenation responses. In the first experiment, two filtered wines received microoxygenation treatments at relatively low rates for 17 weeks, while dissolved oxygen, sulfur dioxide concentration, and acetaldehyde production were monitored. In the second experiment, one wine was divided into filtered

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**Enology — Phenolics Session — CONTINUED**

and unfiltered treatments, with either a low or a high starting sulfur dioxide concentration. Microoxygenation was then administered at a relatively higher rate for eight weeks. Dissolved oxygen, sulfur dioxide, and acetaldehyde were again monitored, along with concentrations of yeast and bacterial populations. These experiments give us insight into how sulfur dioxide concentration, microbial load, and other wine characteristics influence a wine's behavior as oxygen is introduced. The results will help winemakers design better microoxygenation protocols tailored to each individual wine and to monitor the progress of microoxygenation treatments with more precision.

*Funding Support: Constellation Brands*

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**Determination of the Optimum Amount, Rate, and Exposure Point of Microoxygenation for Cabernet Sauvignon**

Anibal Catania, Larry Lerno, Charles Brenneman, and **Anita Oberholster\***

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The controlled addition of small amounts of oxygen to young red wines is known as microoxygenation (MOX). MOX is used during red wine production fairly commonly to increase color density, fruity aromas, and polymeric pigments. Prior research on MOX focused primarily on the comparison of MOX at a single oxygenation rate to non-MOX wines, with the optimum amount and rate of oxygen exposure not being investigated. The amount and rate of oxygenation must be balanced with the wine's specific chemical composition to prevent detrimental effects. To investigate the optimum amount and rate of oxygen exposure, as well as the point of exposure during vinification, a Cabernet Sauvignon was produced at the UC Davis Teaching and Research Winery during the 2014 harvest. Following primary fermentation, the wine was subdivided into two treatment lots, one receiving no oxygen and the other, 20 mg/L/month oxygen for 30 days prior to malolactic fermentation (MLF). After completion of MLF, each treatment was cold-settled and subdivided into treatments that received 0, 4.5, or 9 mg/L/month oxygen for five months. Starting at the end of alcoholic fermentation, all treatments were sampled regularly to determine dissolved oxygen, total and free SO<sub>2</sub>, chromic properties by UV-VIS spectroscopy, phenolic content by RP-HPLC and the Adams-Harbertson Assay, polymeric pigments by LC-MS/MS, and acetaldehyde by RP-HPLC. Descriptive analysis will be performed on bottled wines six months posttreatment (March 2016) with concurrent phenolic analysis. At the end of treatment, wines that received both pre- and post-MLF MOX had more polymeric phenols, polymeric pigments, color density, and hue than the control and wines given only post-MLF MOX. Analysis of the concentration of acetaldehyde during MOX showed little to no buildup during oxygenation.

*Funding Support: Unfunded research*

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## Thursday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology — Phenolics Session — CONTINUED

#### Can Cold Soaking or Massive Sulfur Maceration Prior to Fermentation Increase the Quality of Pinot noir?

**Pascal Wegmann-Herr\*** and Marie Welk

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Compared to other red cultivars, Pinot noir grapes have significantly lower anthocyanin concentrations and more seed tannins and display a general tendency toward unripe tannins. Prefermentative maceration, or cold soaking (CS), aims to lengthen the time of maceration in the absence of alcohol. Another technique gaining popularity among winemakers is adding high sulfur dioxide (SO<sub>2</sub>) concentrations to the grapes to impede the onset of fermentation. However, there are few reports concerning the impact of these techniques on the chemical composition and sensory characteristics of the resulting wines. The aim of this study was to examine prefermentative cold and sulfur maceration as strategies to improve Pinot noir wine quality. Five days of cold soaking at 5°C and SO<sub>2</sub> additions up to 200 mg/L were compared to classical skin fermentations without SO<sub>2</sub> addition and a typical addition of 30 mg/L (control), keeping the total maceration time, fermentation temperature, and punch down frequency identical. Spectrophotometry (color intensity and tone), LC-DAD (total anthocyanins and flavan-3-ols), LC-ESI-ToFMS (Vitisin A and Vitisin B), Harbertson-Adams assays (characterization of phenolic composition) and HS-SPME H/C enantio-MDGC-MS-MS (for  $\alpha$ - and  $\beta$ -ionone and  $\beta$ -damascenone) were used to analyze the differences among treatments. Additionally, a descriptive sensory analysis was conducted with a trained tasting panel. Multiple factor analysis revealed that the cold soak treatments and high sulfur treatments separated well from the control and SO<sub>2</sub>-free wines. Significant increases in color intensity and hue were observed during sensory analysis in both cold soak and high SO<sub>2</sub> addition wines. SO<sub>2</sub>-treated variants lacked fruitiness and exhibited sweaty, sulfur off-flavors. The same wines contained significantly lower  $\beta$ -damascenone concentrations.

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Enology — Phenolics Session — CONTINUED

**Influence of Polysaccharides on Tannin Activity in Red Wine**

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The class of molecules that contributes most significantly to overall red wine astringency is condensed tannins. Due to their molecular mass and surface properties, red wine tannin activity and sensory perception can vary depending on composition, conformation, and interaction with macromolecules present in wine, principally polysaccharides. Polysaccharides from yeasts and/or grapes are released during winemaking and influence tannin perception and red wine stability. High performance liquid chromatography (HPLC) and UV/vis absorbance spectrophotometry were used to improve understanding of tannin behavior in the presence of polysaccharides isolated from Cabernet Sauvignon and Pinot noir wines. After characterization of isolated polysaccharides using size exclusion chromatography, it was determined that Cabernet Sauvignon wines were mostly composed of oligosaccharides, in contrast to Pinot noir wines, which contained an increased proportion of arabinogalactan proteins and mannoproteins. Tannin activity in the presence of polysaccharides, as measured by HPLC, was similar to tannins in the absence of polysaccharides, indicating that polysaccharide interaction with tannins is noncovalent. In contrast, when tannin activity was measured *in situ* using UV/vis absorbance spectrophotometry, activity declined in the presence of polysaccharides. Taken together, the results of this study suggest that it is important to consider both tannins and polysaccharides when investigating red wine astringency and stability.

*Funding Support: American Vineyard Foundation, California State University Agricultural Research Institute*

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# Thursday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

## Viticulture — Water Relations Session

### Grape Berry Transpiration and Its Impacts on Ripening and Weight Loss

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Berry transpiration is important for both berry growth and ripening. Genetically diverse *Vitis* cultivars were used to investigate developmental changes in and factors determining berry transpiration. Transpiration rates were measured on both whole attached clusters (cluster chamber method) and on detached berries (weight loss method). Results obtained using these two methods were in good agreement. The chamber method also demonstrated that detaching berries did not alter their transpiration. The contribution of the rachis to whole cluster transpiration was minor. Berry transpiration rate highly correlated with diurnal changes in vapor pressure deficit, and thus with ambient temperature and relative humidity. Transpiration rate and, to a lesser extent, cuticular conductance, peaked when the berry skin color was red/purple (~13 Brix) and then declined with further ripening. Berries of different cultivars shared similar developmental patterns of transpiration and cuticular conductance, with minor differences in their absolute values. When berry transpiration was artificially reduced in the field, sugar accumulation slowed down yet cracking incidence increased. During late-season ( $\geq 18$  Brix), berry transpiration accounted for a daily loss of 2 to 5% of berry weight across 11 cultivars. These results showed that berry transpiration was determined by both environmental factors (air temperature and relative humidity) and cultivar-specific factors (primarily berry surface area and cuticular conductance). Moreover, berry transpiration facilitated ripening and may partially contribute to late-season weight loss.

*Funding Support: Washington State Grape & Wine Research Program, Washington State University, Chateau Ste. Michelle Distinguished Professorship, Rhone Rangers*

### Thirsty Grapes: Real-time Monitoring of Stem Water Potential

**Michael Santiago,** Winston L. Black, Siyu Zhu, Erik J. Huber,

Adriana C. Mulero, Andrew M. Bryan, Alan N. Lakso, and Abraham D. Stroock\*

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Stem water potential is the best measure of a grapevine's hydration relative to growth, grape yield, and quality; it provides vital information on plant health and helps decide schedules for irrigation and harvesting. Unfortunately, direct measurement of water potential in plant tissue is only possible using labor-intensive, destructive methods such as the pressure bomb and stem psychrometer, and indirect measurements of water potential through the soil using tensiometers are unreliable for woody plants.

We are bringing tensiometers from the soil into the stem. Using microchip technology, we have developed a miniature tensiometer (microtensiometer) small enough to be embedded in the vine stem, where it can measure stem water potential directly from the xylem, in real time, down to potentials below -100 bars without emptying. This sensor has been tested successfully in a laboratory setting and is now being tested in live grapevines. Current development challenges include

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Viticulture — Water Relations Session — CONTINUED

long-term corrosion protection against humidity and UV, mechanical encapsulation, and embedding/sealing protocols. Our goal is to develop a “set and forget” sensor that can provide continuous water potential measurements for multiple seasons without maintenance, allowing vineyards direct control over water status to “dial in” their vintage, conserve irrigation water, and improve grape yield and quality.

*Funding Support: NIFA, USDA, NSF, Sloan foundation*

**Can a Canopy Temperature-based Stress Index Enhance Water Use Efficiency in Irrigated Winegrape under Arid Conditions?**

**Krista Shellie\*** and Brad King

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Enhancement of irrigation water use efficiency and water productivity in arid winegrape production regions is hindered by a lack of automated, real-time methods to monitor and interpret vine water status. A normalized water stress index calculated from real-time vine canopy temperature measurements has been shown in winegrape to correspond well with irrigation amounts and events under arid conditions; however, little is understood about how to interpret water stress index values and the relationship of index values with other commonly used indicators of vine water status. The objective of this study was to relate canopy temperature-based water stress index values for the winegrape cultivars Syrah and Malbec with seasonal measurements of water productivity and carbon isotope composition and to compare daily index values with daily measurements of midday leaf water potential, leaf gas exchange, and leaf chlorophyll fluorescence. Own-rooted vines of each cultivar were grown in replicated plots under arid conditions in an experimental vineyard in Parma, Idaho, and over two growing seasons were supplied with either 35 or 70% of their estimated water demand. The daily water stress index decreased rapidly in response to irrigation events and the amounts of decrease correlated with the amounts of irrigation. In both cultivars, vines irrigated at 35% had higher water stress index values, higher water productivity, and less negative  $^{13}\text{C}/^{12}\text{C}$  in the sugar of mature berries than vines irrigated at 70%. The relationship between the daily stress index and midday leaf water potential was stronger for Syrah than for Malbec. Findings from this study facilitate interpretation of normalized water stress index values and show that canopy temperature is a sensitive indicator of vine water status. Measurement of canopy temperature and calculation of the water stress index is amenable to automation for use as a decision-support tool in a precision irrigation system.

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## Thursday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Viticulture — Water Relations Session — CONTINUED

#### Can Winter Hardiness of Merlot be Improved by Manipulating Irrigation Practices?

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The effects of irrigation deficit timing on the winter hardiness of grapevine buds were determined in a Merlot vineyard in the Okanagan Valley of British Columbia. Treatments were full irrigation (FI) applied throughout the growing season using two emitters per vine, reduced-to-veraison (RV) irrigation, and reduced-to-harvest (RH) irrigation. The latter two were applied from fruitset to veraison or to harvest, respectively, by eliminating one emitter per vine to provide 50% less water. The final treatment, partial root zone drying (PRD), was applied from fruit set to harvest by eliminating one emitter per vine, alternating every three weeks between the two emitter positions at each vine. The frequency and duration of irrigations were the same for all treatments and were based on maintaining soil moisture at 30 to 60 cm deep in the FI treatment at above 4% (v/v). From July to mid-September, leaf gas exchange and stomatal conductance were greater in vines receiving the higher irrigation rate (i.e., FI vines and RV vines postveraison). Higher yields were produced by vines under FI than RV. Compared with FI, all reduced irrigation treatments (RV, RH and PRD) reduced berry weight and increased juice pH but did not affect juice soluble solids or titratable acidity. Bud hardiness measured during the acclimation and maximum-hardiness periods of vine dormancy was lower in response to PRD than all other irrigation treatments and higher for vines that were fully irrigated after veraison than those under the reduced irrigation to harvest regimes. Across treatments, faster rates of photosynthesis, stomatal conductance, and transpiration were associated with enhanced bud hardiness. These results indicate that water stress leading to reduced leaf gas exchange, especially from veraison to harvest, reduces bud hardiness in Merlot.

*Funding Support: BC Wine Grape Council, Agriculture Agri-Food Canada*

#### Irrigation Strategies for White Winegrape Production

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Most knowledge about irrigation management (especially deficit irrigation) has been generated using red winegrape cultivars. Little is known about the optimal irrigation practice for white cultivars. Therefore, three deficit irrigation regimes and a full irrigation treatment (as control) were implemented in commercial vineyards of Chardonnay and Riesling. These treatments were (i) moderate water stress from fruit set to harvest (SDI), (ii) moderate water stress from fruit set to veraison and then no stress through harvest (RDI), (iii) partial rootzone drying from fruit set to harvest (PRD), and (iv) full irrigation (FI). For both cultivars and consistently for two growing seasons, FI vines had higher rates of leaf gas exchange, more canopy growth (vigor, leaf layers, and canopy density), higher yield, and larger berries

**Bold type indicates presenting author**



Viticulture — Water Relations Session — CONTINUED

than SDI or RDI. Compared to SDI, after veraison, RDI vines had increased vine water status and gas exchange rates but no difference in canopy size or yield at harvest. Based on soil moisture measurements, irrigation in PRD was alternated between sides every two to three weeks. In both growing seasons, PRD resulted in a more open canopy and smaller berry size than FI and larger berry size than SDI or RDI. In the second growing season only, PRD increased canopy growth relative to SDI or RDI. Overall, PRD vines had similar berry composition and irrigation water use but lower yield or reduced berry size compared to SDI/RDI; therefore, PRD may be beneficial for white winegrape production. Under similar light conditions, no difference in berry skin flavonoids (flavonols and flavanols) was found between FI and SDI in either sun-exposed or shaded berries. Therefore, light conditions had a dominant impact on berry skin flavonoids compared with irrigation treatments. Irrigation may have indirectly affected skin flavonoids by altering the canopy microclimate.

*Funding Support: WSDA Specialty Crop Block Grant, Washington State Grape & Wine Research Program, University of Milan, COST Action, Innovine European Project*

\*indicates corresponding author

## Thursday National Conference Oral Presentation Abstracts (Research Reports) 2016 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology — Sensory Session

#### **Maceration and Cap Management Affect the Phenolic Distribution and Sensory Profile of Merlot Wine**

**Scott Frost**, Hildegard Heymann,\* John Blackman, Helene Hopfer, Thomas Collins, Jerry Zweigenbaum, Roger Boulton, Susan Ebeler, and Hildegard Heymann

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Cap management and maceration are techniques that manipulate contact between juice and grape solids during winemaking. These practices alter the extraction of phenolic compounds and thus also alter taste and mouthfeel. The effect of four cap management techniques with five postfermentation maceration times was evaluated for phenolic profile and sensory properties. All experiments were conducted in triplicate using Merlot grapes with nine different treatments. Fruit was crushed into 27 200-L fermenters. A three times daily pump-over regime of one juice volume was applied to 18 of the 27 fermenters. At dryness, three of these 18 fermenters were pressed and the remaining 15 fermenters were pressed in groups of three at intervals of one, two, four, six, and eight weeks. Six of the remaining nine fermenters were fitted with a purpose-built wire mesh to submerge the cap into the fermentation. At dryness, three were pressed and three underwent an eight-week maceration and were then pressed. The final three fermenters were punched down three times daily, then pressed when dry. The changes induced by the different winemaking treatments were characterized using instrumental and sensory methods. The sensory profiles of the wines were determined using descriptive analysis (DA) and temporal dominance of sensation (TDS). Hydrophilic interaction chromatography (HILIC) coupled with quadrupole time-of-flight mass detection (qTOF) was used to evaluate the chemical distribution of tannin species. Baseline resolution of proanthocyanidin isomers was achieved, allowing relationships between polymerization and winemaking technique to be observed. Multivariate analysis of the qTOF polyphenol measurements discriminated the nine treatments. Longer maceration times produced proportional increases in specific polyphenol compounds. Extraction patterns and sensory implications will be reported for specific compounds and compound classes.

*Funding Support: American Vineyard Foundation, Stephen Sinclair Scott Endowment*

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Enology — Sensory Session — CONTINUED

**Chemosensory Approach for Understanding the Green, Aggressive, and Hard Character of Red Wines**

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During winemaking, the oenologist must decide how to handle grapes that enter the winery with an acceptable concentration of sugars or acids but with immature tannins. Such grapes generate wines with green, aggressive, or hard (noted as GAH onward) character which influence consumer acceptance of the product. Such decisions are currently based on empirical experience due to the lack of objective criteria. Better chemical and sensory knowledge of immature tannins would allow managing this GAH character efficiently during winemaking. The present work isolates and identifies the group of compounds responsible for the GAH character in wine.

Thirty-eight wines with *a priori* different degrees of GAH were submitted to sensory analysis by a panel of 25 wine experts. Thirteen attributes and two multidimensional terms (preference and GAH) were rated. The GAH concept was correlated negatively to preference and positively to aroma (vegetal) and in-mouth terms (astringency, green, and dry tannins). Four wines with different degrees of GAH were fractionated by solid-phase extraction and semipreparative liquid chromatography. Six odorless fractions (F1 to F6) were isolated from each wine and subjected to characterization of in-mouth sensory properties.

Fractions F3 and F5 induced the highest aggressive character perceived in-mouth among the fractions studied. Fraction F3, which mainly contained anthocyanin-derived compounds with low molecular weight (lower than trimers) and contained an average of 50% of the overall polyphenolic compounds of the original wine, was especially astringent for three out of the four wines studied. In the fourth wine, this fraction was mainly described as “sticky.” Fraction F5 contained polyphenols of high molecular weight (higher than trimers) and, on average, ~15% of the overall polyphenolic content of the original wine. This fraction was especially astringent and *dry* for the wine scored highest in GAH. These results are promising and suggest that the developed chemosensory approach succeeded in isolating a group of compounds potentially involved in the green, aggressive, and hard character of wines.

*Funding Support: Spanish Ministry of Economy and Competitiveness (MINECO) and the European Union (FEDER Funds)*

\*indicates corresponding author

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## Enology — Sensory Session — CONTINUED

### **White Juice Solids: Effect on Phenolics, Polysaccharides, and Mouthfeel of White Wine**

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Fermenting white juices containing grape solids may contribute to the mouthfeel and therefore the overall complexity of white wine. Chardonnay wines were made from juices containing grape solids (unclarified, 500 ntu and 100 ntu) by cold settling using enzymes, bentonite, and without a settling agent. Other wines were produced on a commercial scale from Chardonnay, Riesling, Viognier, and Sauvignon blanc from unclarified and clarified (200 ntu) juices. The effects of the degree of juice clarity and the method used to achieve it on wine phenolic and polysaccharide composition were determined. Greater juice solids resulted in higher concentrations of wine polysaccharides, which contained a greater proportion of mannoproteins and arabinogalactan proteins. Juice solids content did not consistently affect total wine phenolic concentration; however, the relative contribution of phenolic types to the total varied significantly. Wines from high-solids juices were perceived by a trained texture panel to be more “oily” and “metallic” than those produced from clarified juices. Possible relationships between a wine’s mouthfeel attributes and its phenolic and macromolecular composition are discussed.

*Funding Support: Wine Australia*

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## Enology and Viticulture

### Optimizing Genetic Transformation of Grapevine Fruiting and Rootstock Cultivars

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A protocol was standardized to regenerate six grapevine cultivars through meristematic bulk (MB) induction, which was used for genetic transformation. Meristematic bulk induction worked best with *Vitis vinifera* Thompson Seedless (98.4%), followed by Chardonnay (97.6%), Redglobe (90.2%), and Cabernet Sauvignon (86.2%). It was less successful with *V. rupestris* St. George (85.4%) and *V. riparia* x *V. rupestris* 101-14 Millardet et de Grasset (79.6%). Benzylaminopurine (BA) and naphthaleneacetic acid (NAA) was the most effective combination of cytokinin and auxin for MB formation. Kanamycin was a better antibiotic selection agent than hygromycin during transformation. The expression of green fluorescent protein (GFP) was evaluated with *in vitro* leaves and roots. Transformation efficiency using meristematic slices was a function of genotype. Transformation efficiency was greatest in Chardonnay (51.7%), followed by Thompson Seedless (42.3%), St. George (41.6%), Redglobe (40%), Cabernet Sauvignon (35.6%), and 101-14 Mgt (29.9%). Overall, MB induction was a faster and simpler alternative for genetic transformation of grapevine cultivars.

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### Evaluation of Native Non-Saccharomyces Yeasts for Reducing Ethanol Production in Wine By Sugar Respiration

**Jesse Aplin**, Charles Edwards,\* Nicholas Rivera, and Dean Glawe

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Due to changes in viticultural practices coupled with increasing temperatures in growing regions, sugar concentrations in grape musts have increased, leading to increased ethanol concentrations in the resulting wines. A natural component of the grape berry microbiome, non-*Saccharomyces* yeasts have the potential to reduce ethanol yield through respiration of glucose/fructose. The objective of this research was to identify native yeast strains that do not express the biochemical Crabtree Effect whereby sugars are fermented, not respired, regardless of oxygen availability. Non-*Saccharomyces* yeasts were screened using a high Brix Merlot grape juice (310 g/L 1:1 mixture of glucose/fructose and 270 mg/L yeast assimilable nitrogen) with and without aeration. After six days, aliquots were inoculated with a commercial strain of *Saccharomyces cerevisiae* to finish fermentation. While most aerated fermentations consumed less sugar and more nitrogen than nonaerated ones, some isolates also yielded less ethanol. Native strains of *Candida* spp.

\*indicates corresponding author

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### Enology and Viticulture – CONTINUED

exhibited the greatest ethanol reduction under aerated conditions but accumulated excessive amounts of acetic acid that will limit industrial application. The native *Metschnikowia pulcherrima* isolate is more promising, giving a similar reduction in ethanol concentration without excessive acetic acid production. Though screening results are encouraging, this study shows that aeration and available nitrogen must be optimized to reduce potential deleterious effects on the wine's sensory profile prior to industry application.

*Funding Support: Wine Advisory Committee of the Washington Wine Commission, Agricultural Research Center, WSU-CAHNRS*

### Vineyard Irrigation Scheduling based on Remote-Sensed Estimates of Vine Water Status Using Aerial Thermal Images

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Some components of berry composition depend on vine water status history during the growing season. The effects of timing, severity, and duration of water stress differ among varieties. Water stress indicators are therefore valuable to determine the most appropriate irrigation strategy for each variety. A further difficulty comes from the extreme spatial heterogeneity of vine water status within a vineyard, which can limit the practical implementation of irrigation management. A grower wanting to decrease the variability of within-vineyard berry composition and yield by managing irrigation more effectively must measure and manage the spatial variability of vine water status, which constitutes a major challenge. Remotely-sensed thermal imagery is a powerful tool used in precision viticulture to provide accurate geospatial maps of variability in vine water status. The crop water stress index (CWSI), derived from relating the canopy leaf to air temperature difference with air vapor pressure deficit, has been developed for different grapevine varieties and seasonally related to leaf water potential ( $\Psi_{\text{leaf}}$ ). A high-resolution thermal sensor installed onboard an aircraft is being used in Spain to remotely estimate  $\Psi_{\text{leaf}}$  at the scale of individual vines. Thus, the average leaf of individual irrigation sectors can be used as an irrigation trigger. The optimal pixel size, time of the day for image acquisition, starting day of the season, image acquisition frequency, and turnaround time have also been determined. Currently, researchers in Spain can fly over and gather thermal images from 1,500 hectares daily at 30-cm pixel size resolution and deliver irrigation recommendations for individual irrigation sectors according to vine water status ( $\Psi_{\text{leaf}}$ ). In collaboration with UC Davis, this technology is being implemented in California to provide irrigation recommendations based on remote-sensed estimates of vine water status.

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Enology and Viticulture – CONTINUED

**Aromatic Variables Associated with the Quality of Texas High Plain Wines Made from Tempranillo and Cabernet Sauvignon**

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Sensory analysis of wine has become of increasing importance to understand aromatic properties and their link to quality perceived by consumers. We assessed the sensory profiles of wines made from Tempranillo and Cabernet Sauvignon grapes grown in the Texas High Plains. We used conventional descriptive analysis to associate the quality and intensity of aromatic families perceived in those wines by a panel of Texas High Plains red wine consumers and to differentiate the two types of varietal wines. The panelists were asked to rate global smell and taste aromatic intensities, and for each category, the intensity of five aromatic families: fruit, floral, vegetal, spice, and wood. They were also asked to rate “balance” components: alcohol, sweetness, acidity, and tannins. Finally, we asked them to rate the perceived quality of each wine. We used principal component analysis to check for correlation between aromatic variables and quality. There was no grouping of Tempranillo or Cabernet Sauvignon wines using those aromatic variables. The global intensity of smell and taste correlated with the intensity of wood and spice aromas. The quality perceived by the panel was also associated with global intensity and spice and wood aroma. Based on those results, we conclude that the panel of red wine consumers associated quality of Texas High Plains wines made from Cabernet Sauvignon or Tempranillo with global smell and taste intensity and intensity of oak aging-related aromas (spice and wood), and not with varietal-related aromatic families (fruit, floral, and vegetal). The focus on oak aging aromatic families and their association with global intensity could explain the panel’s lack of ability to distinguish the two types of varietal wines, as those aromatic variables were more discriminant than varietal-related aromatic families.

*Funding Support: Texas Tech University*

**Response of Different Grape Rootstock and Cultivar Combinations to Incidence, Severity, and Defoliation Caused by Downy Mildew**

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Downy mildew (*Plasmopara viticola*) is an important disease in grapes worldwide. Disease control is based on preventive management, including reduction of overwintering inoculum, green pruning, use of less susceptible cultivars, and fungicide application. The objective of this study was to describe the incidence, severity, and defoliation of *P. viticola* development in three cultivars, Concord, Bordô, and BRS Carmem grafted onto Paulsen 1103, IAC 766, and VR 043-43 rootstock on a commercial plantation in a subtropical area. To compare the response of each cultivar and rootstock combination, the experiments were carried out on ridges with a semi-trellis conduction system. Disease incidence and severity were assessed every 14 days from September to March 2011/2012 and 2012/2013 on the last 10 leaves on specific shoots of six plants of the nine different combinations. During the growing season, the weather conditions were favorable for development

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### Enology and Viticulture – CONTINUED

of the disease. Symptoms started in the vegetative phase with continuity during fruiting. There were differences among cultivars with BRS Carmem being most susceptible to mildew. Rootstock had no influence on downy mildew severity; however, the combinations showed the great influence of phenology on downy mildew. Defoliation influenced final production and phenology. Thus, the choice of less susceptible cultivars should be taken into consideration regarding phenology.

*Funding Support: CAPES; CNPq*

#### Growing Region and Winery Influences on the Sensory Quality of Merlot Wines from the Okanagan and Similkameen Valleys

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The influences of terroir and winery practices on the sensory character of Merlot wines produced in the Okanagan and Similkameen valleys in British Columbia were evaluated in four vintage years: 2004, 2005, 2012, and 2013. The wines were produced from grapes grown in six regions that differ in climate, soil, and topographic characteristics. Two to nine single-vineyard wines per region were acquired from commercial wineries each year. Wineries that provided more than one wine had sourced the fruit for each wine from a different region. Sensory quality of the wines was characterized ~20 months after the wines were made. The wines were blindly tasted by 31 judges who each tasted 10 wines twice in a balanced incomplete block design. The intensity of nine aroma and nine flavor and mouthfeel characteristics were rated. These included floral, fruit, vegetative, and herbaceous flavors and aromas, tannin roughness, body, and length of aftertaste. The sensory characteristics that distinguished the wines of each region and each winery that provided multiple wines were identified by discriminant function analysis. Although regional and winery characteristics differed among vintage years, some consistencies were found. Wine sensory characteristics were acquired from both terroir and winery influences in each vintage year.

*Funding Support: BC Wine Grape Council, BC Wine Institute Agriculture, Agri-Food Canada*

#### Evaluation of Riesling Clonal Selections in the Salinas Valley

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Eleven clonal selections of Riesling were evaluated for viticultural performance for three years (2013 to 2015). The trial design was a randomized complete block with eight replications of five vine plots. Riesling FPS selections 1 (21B from OSU), 4 (CA), 9 (Gm 110), 10 (CA, Martini), 12 (Neustadt 90), 17 (Gm 198), 20 (Alsace from CA), 21 (Neustadt 365), 22 (Mendoza, Argentina), 23 (Gm 239-25), and ENTAV 49 were field-budded in 2008 onto 101-14 Mgt. rootstock

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Enology and Viticulture – CONTINUED

planted in 2007 at a vineyard site southwest of Soledad (Arroyo Seco American Viticultural Area). Vines were planted at a row and vine spacing of 2.4 × 1.5 m, trained as unilateral cordons, and spur-pruned on a vertical shoot-positioned trellis. Significant differences were observed in yield, with a range of 1.29 kg/vine from high- to low-yielding selections. Riesling selections 1, 9, 12, 23, and 22 had the highest yields; 17 and 4 were intermediate; and 10, 49, 20, and 21 had the lowest yields. Higher cluster weights were the factor most influencing crop yield. Either more berries per cluster or greater berry weight increased cluster weight. Although not significant, pruning weights had a range of 0.27 kg/vine from high to low weights among the selections. Yield:pruning weight ratios were greater for the more productive selections. They ranged from 7.6 (1) to 4.5 (20). There were significant differences among selections in fruit composition at harvest: higher-yielding selections had lower Brix and pH and higher titratable acidity.

*Funding Support: no external funding*

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**Analysis of *Brettanomyces bruxellensis* Penetration Depths in Different Types of Oak Barrel Staves**

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*Brettanomyces bruxellensis* is a yeast often associated with spoilage during barrel aging of wines. It is therefore important that the wine industry develops more efficient oak sanitation protocols to avoid contamination by this microorganism. This requires a consensus regarding to what depths this yeast can be found in barrel staves. The penetration of *B. bruxellensis* into oak staves with different oak type, toasting level, and physical location within a barrel was studied. For the experimental design, 16-L barrels made from French or American oak (*Q. petraea* or *Q. alba*, respectively) and processed with two different toasting levels were obtained. The barrels were infected with *B. bruxellensis* for seven months with one un-inoculated barrel of each type serving as controls. Once emptied, the barrels received a hot water/SO<sub>2</sub> rinse before being disassembled. Individual staves were cut into 3 x 10 cm blocks before being subdivided into four mm thick layers (inside to outside of barrel). These layers were placed in a sterile-filtered, nutrient-enriched wine to recover any viable cells present. Additional layers were observed under scanning electron microscopy (SEM) to compare oak structure and yeast infiltration. Key findings included differences in *B. bruxellensis* recovery from the two types of oak studied. The furthest penetration occurred in French oak staves located at the bottom of barrels between five to nine mm. Additionally, yeast populations from heavy toasted staves tended to enter logarithmic growth more quickly. SEM images agreed with the recovery data and suggested possible pseudohyphae formation by this yeast. Our research demonstrates that *B. bruxellensis* oak contaminations differ with respects to oak species, toasting level, and stave location. Knowing the depths of yeast infiltration, better protocols to eliminate *B. bruxellensis* from contaminated barrels can now be investigated.

*Funding Support: Wine Advisory Committee of the Washington Wine Commission, Agricultural Research Center, WSU-CAHNRS*

\*indicates corresponding author

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### Enology and Viticulture – CONTINUED

#### **Sunburn in Grape Berries: Varietal Differences in Composition, Structure, and Physiology**

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A major concern during grape ripening is sunburn, which can be a significant problem for winegrape production worldwide and in eastern Washington. Commercial grapevine cultivars differ in heat tolerance; this study characterizes tolerance to sunburn among red (Merlot, Syrah, Cabernet Sauvignon, Grenache, Sangiovese, and Cabernet Franc) and white (Riesling, Chardonnay, and Gewurztraminer) cultivars by analyzing skin characteristics and fruit composition including Brix, pH, TA, malic and tartaric acids, glucose and fructose, potassium, YAN, and phenolic compounds including total anthocyanins, anthocyanin profile, and tannins. The symptomatology of sunburn varied between red and white cultivars: in red cultivars, color development was poor, while brown lesions appeared on the skins of white cultivars. In all cultivars, the exposed surfaces lost the crystalline structure of epicuticular wax, resulting in a shiny surface. The wax platelets were degraded and transitioned into amorphous masses, creating a rough surface morphology. The flesh cells incurred loss of cell viability. Generally, sunburned berries of all cultivars dehydrated, increasing Brix, but acids were lost to respiration. The degree of quality deterioration due to sunburn varied and was a function of cultivar, which is linked to berry structure and physiology.

*Funding Support: Washington State University*

#### **Microwave-assisted Extraction of Cabernet Sauvignon Grapes: Effects on Wine Phenolics, Color, and Sensory Properties**

**Federico Casassa**,\* Santiago Sari, Mariela Diaz-Sambueza, Esteban Bolcato, and Vanesa Garcia

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Microwave-assisted extraction (MW) is scalable to industrial conditions and thus can be adopted by the wine industry. Cabernet Sauvignon grapes with a marked vegetal character were submitted to MW (seven min, peak temperature  $53 \pm 3^\circ\text{C}$ ,  $n = 24$ ). Untreated control wines were also produced. All wines were produced in triplicate with 12 days maceration and were followed for phenolics and color from pressing up to three months of bottle aging (BA). MW reduced total yeast counts. Ethanol, titratable acidity, malic acid, lactic acid, dry extract, and glycerol were more concentrated in MW wines. Extraction of anthocyanins, tannins, and total phenolics at pressing was 36, 26, and 27% higher in MW wines. After three months of BA, anthocyanins declined 42% in MW wines and 21% in control wines; tannins declined 45% and 29%, respectively. As a result, after three months BA, there were no differences between MW and control wines for anthocyanins, tannins, total phenolics, color hue, or polymeric pigments, but wine color (AU  $420 + 520 + 620 \text{ nm}$ ) remained higher in MW wines. Descriptive sensory analysis with a trained panel ( $n = 21$ ) found that MW wines had greater color satura-

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**Enology and Viticulture – CONTINUED**

tion, purple color, fruity aroma, astringency, and bitterness than control wines. Additionally, MW reduced the vegetal character of the MW wines. Overall, MW applied to Cabernet Sauvignon grapes had a positive effect on phenolic extraction that was observable early during winemaking, but the rate of loss of phenolics in MW wines was higher than in control wines. Although the long-term phenolic and chromatic composition of MW was not significantly altered relative to a control, MW was a viable option to manage the vegetal character of Cabernet Sauvignon wines while positively altering mouthfeel and taste properties.

*Funding Support: INTA*

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**Cold Soak to Reduce Maceration Length: Chemical, Chromatic, and Sensory Effects in Cabernet Sauvignon, Malbec, and Merlot**

**Federico Casassa,\*** Esteban Bolcato, Vanesa Garcia, Santiago Sari, Mario Gallina, Maria Jose Miranda, and Nora Barda

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Prefermentation cold soak (CS), is sometimes applied to improve anthocyanin extraction while limiting tannin extraction during ensuing maceration. Cabernet Sauvignon, Malbec, and Merlot grapes were processed with CS (five days at  $7 \pm 2^\circ\text{C}$ ) followed by both a short maceration (five days, CS + 5d) and a long maceration (10 days, CS + 10d) and contrasted against a control wine (10-day maceration). Wines were elaborated in triplicate ( $n = 3$ ) in 100-L stainless steel fermenters. Phenolics and color components were analyzed in the finished wines and the Malbec and Merlot wines were submitted to descriptive sensory analysis. The maceration techniques had no impact on residual sugars, volatile acidity, titratable acidity, or pH. A two-way ANOVA on the phenolic composition uncovered a significant effect of cultivar on all phenolic classes. Across the three varieties, anthocyanins were more abundant in CS + 5d and CS + 10d wines, while tannins were more abundant in control and CS + 10d wines. Analysis of absorbance in the visible spectrum indicated that in Malbec and Merlot, control and CS + 5d wines achieved comparable absorbances at 420, 450, 520, 570, 620, and 630 nm, but CS + 10d had less absorbance. However, in Cabernet Sauvignon, CS + 5d wines had the least absorbance. Descriptive sensory analysis with a trained panel ( $n = 12$ ) uncovered significant differences in color intensity, red fruit aroma, bitterness, and body in Malbec wines and overall, red fruit, and dried fruit aromas, sweetness, astringency, dryness, and length in Merlot wines. Overall, CS + 5d reduced bitterness, astringency, and dryness. Thus, CS followed by a short maceration time (five days) limited tannin extraction while maintaining or moderately improving selected chromatic and aromatic features relative to a control wine.

*Funding Support: INTA*

\*indicates corresponding author

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### Enology and Viticulture – CONTINUED

#### Microwave-assisted Extraction of Merlot Grapes at Different Stages of Ripeness

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Microwave-assisted extraction (MW) is scalable to industrial conditions and thus potentially could be adopted by the wine industry. Merlot grapes harvested at three different stages of ripeness (21, 24.5, and 28 Brix) were subjected to MW (seven min, peak temperature  $50 \pm 4^\circ\text{C}$ ,  $n = 48$ ) and made into wine. Additional, untreated wines were produced as a control. All wines were produced in triplicate with a 12-day maceration and were followed for phenolics and color from pressing to five months of bottle aging (BA). MW had no effect on the basic composition of the wines (alcohol, residual sugars, glucose, fructose, titratable acidity, malic, citric, lactic and acetic acids, pH, and glycerol) with the exception of tartaric acid concentrations, which were lower in MW wines. Extraction patterns of anthocyanins, tannins, polymeric pigments, and wine color indicated that MW applied to unripe fruit (21 Brix) increased all of these parameters at pressing and the differences persisted after five months of bottle aging. In ripe (24.5 Brix) and overripe fruit (28 Brix), MW only enhanced tannins by ~11%, but in unripe fruit, MW enhanced tannins by 27% after five months. For the three ripeness stages, MW achieved a quick extraction of monomeric anthocyanins, averaging a 193% increase relative to their controls, but these differences were reduced and disappeared in wines made from ripe or overripe fruit. The wines and their replicates were also analyzed for monomeric anthocyanins, pyranoanthocyanins, and flavanol-anthocyanin adducts by HPLC-DAD-MS after malolactic fermentation. Total anthocyanins, total glucosides, coumaroyl-glucosides, cinnamoyl-glucosides, B-type vitisins, and total pyranthocyanins increased progressively in the wines as fruit ripeness increased from 21 to 28 Brix. MW had no effect on total anthocyanins but increased acetyl-glucosides, B-type vitisins, and flavanol-acetyl-anthocyanin adducts, the latter probably due to their higher tannin concentration. In Merlot, MW could be a valuable tool to treat unripe fruit to enhance phenolic extraction and color stability over time.

*Funding Support: INTA*

#### Effect of Water Availability and Rootstock on Chambourcin Vine Physiology and Grape Quality

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As water availability decreases and human demand for water increases, it is necessary to minimize vineyard water use while maintaining wine quality and yield. To assess rootstock and irrigation effects in tandem, a vineyard was established in 2008 in Mt. Vernon, Missouri. Six irrigation zones were installed, allowing randomization of blocks for three different irrigation regimes and four different rootstocks. Chambourcin vines, either own-rooted or grafted onto 1103P, SO4,

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or 3309C, were planted and either (i) nonirrigated, (ii) irrigated at full replacement of evapotranspiration (ET), or (iii) irrigated at 50% of the potential ET (RDI). From 2013 to 2015, soil moisture, leaf gas exchange, leaf chlorophyll content, canopy density, yield metrics, and vegetative growth were monitored and harvested fruit was vinified. In the unirrigated and RDI treatments, the drought decreased mean volumetric soil water content. Midday leaf water potential indicated varying tolerance of water stress, with own-rooted vines showing greater stress under all three irrigation regimes than vines grafted onto SO4 rootstock. Photosynthetic carbon assimilation rate was reduced in nonirrigated and RDI vines on all rootstocks. There were positive correlations between midday mean volumetric soil water content and leaf water potential, leaf stomatal conductance, and leaf transpiration rate. Nonirrigated vines had significantly altered canopy development, a reduced percentage of interior clusters, and increased cluster flux availability and flux symmetry. Treatments influenced fruit composition at harvest with differences in berry weight: in vines grafted onto SO4, full irrigation produced larger berries than both other grafted and own-rooted vines. Differences in fruit composition included higher juice soluble solid content in fruit from own-rooted, nonirrigated vines than in fruit from SO4-grafted, fully irrigated vines. Also, higher juice pH was measured in fruit from 1103P-grafted, partially irrigated vines than in fruit from own-rooted vines.

Funding Support: Missouri Wine and Grape Board

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**Potential Impact of Red Blotch Incidence on Wine Composition and Wine Quality in Cabernet Sauvignon**

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The potential impact of Red Blotch on wine quality will likely depend upon incidence in the vineyard. At low incidence, the impact may be negligible; however, at higher incidence the impact may be substantial. The objective of this study was to determine the threshold incidence of Red Blotch in Cabernet Sauvignon vineyards at which separation of harvest is required to avoid negative impacts on wine quality. Small-lot wines were made from Cabernet Sauvignon grapes from the same vineyard block with 0, 25, 50, or 100% of fruit from Red Blotch-infected vines. Must composition (Brix, pH, and TA) correlated significantly with percent Red Blotch fruit. Wine composition was also impacted significantly by percentage Red Blotch fruit. Ethanol correlated negatively ( $r^2 = 0.97$ ) with percent Red Blotch fruit. Wine color was impacted by percent Red Blotch fruit. Color density ( $A_{420} + A_{520}$ ) correlated negatively ( $r^2 = 0.92$ ). Total iron-reactive phenols ( $r^2 = 0.60$ ) and tannins ( $r^2 = 0.67$ ) in finished wines correlated negatively with percent Red Blotch fruit in must. The results show that the potential impacts of Red Blotch on must and wine composition depend upon incidence in the vineyard and degree of fruit maturity delay in virus-infected vines. Duo-trio difference tests were performed on wines. There was a significant difference between wines made with 0 and 50% Red Blotch fruit ( $p < 0.05$ ). The comparison of wines with 0 and 25% Red Blotch fruit was inconclusive ( $p = 0.059$ ). There was no significant differ-

\*indicates corresponding author

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ence between wines with 25 and 50% Red Blotch fruit ( $p = 0.40$ ). Incidence of Red Blotch from 25 to 50% are sufficient to have an significant impact on wine sensory properties and, therefore, quality. Separate harvest of virus free and Red Blotch vines is likely warranted when vineyard incidence of Red Blotch is above 25%.

*Funding Support: Treasury Wine Estates*

### Performance of UC Davis-Developed, Pierce's Disease-Resistant *Vitis vinifera* L. Selections in Alabama

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Pierce's disease (PD), caused by the bacterium *Xylella fastidiosa*, is the major limiting factor for production of *Vitis vinifera* grapes in the southeastern United States. Three PD-resistant *V. vinifera* selections developed at UC Davis, 501-12, 502-01, and 502-10, were planted at the Chilton Research and Extension Center, Clanton, in 2010 to study their resistance to PD and overall performance in an environment with high PD pressure. The experimental vineyard is a RCBD with six blocks and five vines/block. No PD infection was detected during plant establishment. Studies on vine phenology, vegetative development, and cropping potential were initiated in 2015. Selections 502-10 and 502-01 started their development two to four days prior to 501-12. All selections initiated budbreak at the end of March. Vines had fully-developed canopies by April 20. Selection 502-01 had the earliest flowering season; however, all selections reached full flowering by May 5. All selections grew vigorously during the years of vineyard establishment. Fruit of selection 502-10 matured early in the season and was harvested in mid-August, followed by 502-01 at the end of September to early October. The selection 501-12 ripened late and was ready to harvest in the second half of October. The selections differed in yield per vine, but all were highly productive. Cumulative yield per vine for 2012 to 2015 was greatest (28 kg/vine) for the late-season 501-12, while the early- and mid-season selections produced 25.2 and 23.8 kg/vine, respectively. Early-season selection 502-10 had the largest cluster size among the group: 467 g on average in 2015. Based on six years of observations, the study results are encouraging. The newly introduced PD-resistant grape selections have the potential to improve grape production sustainability in the southeastern region and enhance the agriculture and food systems.

*Funding Support: Auburn University, AL*

### Polysaccharide Release by Immobilized *Schizosaccharomyces japonicus* Cells during Alcoholic Fermentation

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Yeasts belonging to the genus *Schizosaccharomyces* have been proposed for use in wine fermentation because of their ability to metabolize malic acid, permitting

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non-bacterial biological deacidification and averting production of amines. However, *Schizosaccharomyces* yeasts are associated with the presence of off-characters when left too long in the wine after malo-alcoholic fermentation. To overcome this problem, cells of *S. pombe* were immobilized in calcium alginate, used in mixed fermentation with *Saccharomyces* yeasts, and removed once the desired malic concentration was achieved. Non-*Saccharomyces* yeast confer other benefits such as the release of polysaccharides that can improve wine quality.

A previous screen of *Schizosaccharomyces* strains with different malic acid consumption patterns identified those with high polysaccharide release. A strain of *Schizosaccharomyces japonicus* showed the greatest polysaccharide release. Here, we evaluated the release of polysaccharide during alcoholic fermentation by an immobilized version of that strain. A commercial strain of *S. cerevisiae* was inoculated simultaneously and sequentially (after 48 hours) with immobilized cells of *S. japonicus*. The high release of polysaccharide by the immobilized *Schizosaccharomyces* strain was confirmed. The greatest polysaccharide release was observed in the sequential trials. The influence of the inoculation mode on the interactions between the two yeast strains and on the analytical profiles of the final wines was also evaluated. Our findings suggest that immobilized cells of *S. japonicus* not only reduce acidity, but also increase the concentration of active polysaccharide, with beneficial effects for the wine.

*Funding Support: Dipartimento di Gestione Sistemi Agrari, Alimentari e Forestali (GESAAF), Università degli Studi di Firenze, 50144 Firenze, Italy*

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**Rootstocks for Management of *Meloidogyne hapla* in Washington State Vineyards**

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The plant-parasitic nematode *Meloidogyne hapla* (northern root-knot) is damaging to *Vitis vinifera* in the Pacific Northwest (PNW). While nematode-resistant rootstocks are available, they are rarely used in the PNW. Previous greenhouse studies indicate that rootstocks are poor hosts for *M. hapla* compared to own-rooted *V. vinifera*. To further explore the use of rootstocks for *M. hapla* management, two additional trials were conducted. In the first trial, six rootstocks (101-14 Mtg, Harmony, 1103P, Teleki 5C, and own-rooted grafted and ungrafted Chardonnay) were planted in spring 2015 into either fumigated or nonfumigated areas of a vineyard in Patterson, Washington. In nonfumigated areas, a subset of vines was inoculated with *M. hapla*. Soil was sampled in the spring and fall for *M. hapla*. Fumigation initially controlled *M. hapla*, but populations began to rebound by October 2015. Own-rooted Chardonnay in nonfumigated, inoculated plots had significantly higher *M. hapla* population densities than in the fumigated plots. Rootstocks 101-14, 1103P, and 5C did not have statistically different nematode population densities between fumigation treatments, but Harmony had significantly more *M. hapla* in inoculated treatments. The second trial was conducted in the greenhouse. Four rootstocks (Harmony, Riparia Gloire, 3309C, and St. George) and an own-rooted Chardonnay control were challenged with four popu-

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lations of *M. hapla* originally collected from vineyards in Oregon and Washington. Potted vines were inoculated with three *M. hapla* eggs/g soil. At the end of the experiment, vines were assessed for eggs per gram root and reproductive factor. Own-rooted Chardonnay had a high reproductive factor (50), while all rootstocks had reproductive factors below 1. This low reproduction factor indicates that these rootstocks are poor host for *M. hapla* populations. These combined results suggest that if rootstocks are considered in the PNW, selecting those with resistance to *M. hapla* may improve management options.

*Funding Support: Washington State Wine and Grape Research Program*

### Assessing Yeast Communities within Inoculated and Spontaneous Fermentations by Culture-Dependent and Independent Methods

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The Okanagan valley of British Columbia, Canada, includes over 130 wineries. This has caused Okanagan winemakers to seek to produce a more unique product by means of spontaneous fermentations. Traditionally, inoculated fermentations are conducted by adding an abundance of a particular *Saccharomyces cerevisiae* yeast strain to produce consistent and specific chemical and sensory attributes in wines; however, many winemakers are choosing to conduct spontaneous fermentations to achieve more complex characteristics in their wine. Our first objective was to compare *S. cerevisiae* strain and yeast species diversity and composition between inoculated and spontaneous fermentations of Pinot noir and Chardonnay musts. Our second objective was to determine whether yeast species assemblages, diversity, and composition differed between culture-dependent identification methods using microsatellites and DNA sequencing and culture-independent identification via the Illumina MiSeq platform. Using the culture-dependent method, uniquely different *S. cerevisiae* populations and yeast communities were found between spontaneous and inoculated fermentations of Pinot noir; however, there was no difference between the two fermentation treatments in Chardonnay. Unexpectedly, both Pinot noir and Chardonnay fermentations included non-*Saccharomyces* yeasts, specifically *Hanseniaspora* and *Pichia* sp., throughout fermentation and in large relative abundances. Culture-dependent or independent analyses had similar results when describing species diversity and composition. The detection by both methods of unique yeast species assemblages and differences in diversity and community structure between inoculated and spontaneous fermentations of Pinot noir musts indicate that both methods are valid. However, the culture-independent method represented a microbial community based on the whole sample rather than a portion of the sample as in the culture-dependent method.

*Funding Support: BC Wine and Grape Council, NSERC*

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**Antioxidant Capacity of Table Grapes from the South San Joaquin Valley is Related to Skin Color**

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and **Sarah Forester\***

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Kern County, located in the South San Joaquin Valley, is the fourth largest agricultural-producing county in the United States and grapes are its largest commodity, followed by almonds and milk. Grapes contain polyphenols that have health-protective effects, including prevention of heart disease and cancer. The protective effects of grape polyphenols have been associated with their antioxidant activities. Additionally, darker coloration in the flesh and skin of fruit, such as orange, red, or black, is associated with enhanced antioxidant activity. The ORAC (oxygen-radical absorbance capacity) assay is widely-used to quantify the antioxidant activities of grape polyphenols. In this study, seven commercial and experimental table grape varieties were selected with a range of skin colors: pale green, dark green, pale red, medium red, dark purple, and black. The skins, seeds, and pulp were separated and extracted with both water and acetone. From these samples, the antioxidant capacity as ORAC of the different fractions and grape varieties were compared. Total phenols (gallic acid equivalents) of all fractions were also determined and correlated well with the ORAC values. This study provided ORAC values for South San Joaquin Valley table grapes and also confirms that these values correlate well with total phenolic concentration.

Funding Support: Grapes and some chemical supplies provided by Sun World International, LLC. Additional funding provided as start-up funds for Dr. Forester at CSUB.

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**Assessing the Effect of Different Percentages of Red Blotch-Affected Fruit on the Composition of Cabernet Sauvignon Wine**

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As Grapevine Red Blotch-associated Virus (GRBaV) has only recently (since 2011) been identified as the causal agent of Red Blotch (RB) disease, there is a general lack of scientific knowledge regarding how grape and wine composition and quality are affected. RB can severely affect sugar accumulation and color development in red grapes. To deal with RB, growers and winemakers must react based on the severity and incidence of the disease. Possible actions include selected harvesting or replanting the affected vineyard. The objective of this study was to provide the industry with some insight into how inclusion of RB-affected grapes will impact wine quality. Cabernet Sauvignon grapes from infected (RB(+)) and uninfected (RB(-)) vines were harvested separately from a vineyard in Rutherford, CA, in 2015. Wines were made in triplicate from fruit containing different percentages of RB(+) fruit (0, 5, 15, 25, or 100%) by mass. Basic wine chemistry

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(ethanol, volatile acidity, titratable acidity, pH, and free and total SO<sub>2</sub>) and phenolic profiling of the wines was performed by ETS Laboratories to determine the impact of including RB(+) fruit on wine composition. Phenolic profiling showed significant differences between RB(+) and RB (-) wines in the concentration of many phenolic compounds, including monomeric flavan-3-ols, anthocyanins, and polymeric pigments, but no significant difference in tannin concentration. Wines will also be analyzed by phloroglucinolysis to investigate potential differences in tannin composition and by HS-SPME-GC-MS to determine the aroma profiles. Formal descriptive analysis of the wines will commence shortly. Preliminary evaluation of the wines indicated clear differences among wine treatments in both flavor and mouthfeel.

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### **Foliar Spray Application of Inactive Dry Yeast at Veraison: Effect on Berry Skin Thickness, Aroma, and Phenolic Quality**

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Achieving a satisfactory aroma and phenolic maturity at harvest is key to producing quality wines. A foliar spraying treatment with yeast derivatives was tested on the grape (*Vitis vinifera* L.) varieties Chardonnay, Cortese, Barbera, and Nebbiolo. The treatment was carried out at veraison with two different formulations for white and black varieties (LalVigne Aroma and LalVigne Mature, respectively) to enhance aroma and phenolic quality. The influence of the treatments on berry skin thickness was also evaluated. The analyses were carried out on grapes at harvest and experimental wines were produced and analyzed. The berry distribution of Chardonnay and Cortese grapes in density classes, obtained by flotation in saline solutions, evidenced smoother ripening of treated berries and resulted in an average increase in must acidity without affecting sugars accumulation (Brix). Furthermore, berry skin thickness also increased in treated berries. This textural result was also found in Nebbiolo, while Barbera did not show a clear difference. Skin phenolic quality, evaluated by maceration in wine-like solutions, of Barbera was not significantly affected, probably because this variety is characterized by low skin flavanol concentrations. Instead, Nebbiolo evidenced a positive influence of the treatment in extracted and total anthocyanins and flavanols. Overall, the treatments influenced the grapes quality, providing a tool to winemakers for differentiation of the products.

*Funding Support: Università degli Studi di Torino*

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**Chemistry of Red Wine Aging: Implications for Health and Biologic Activity**

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Polyphenols are a large and complex group of bioactive compounds responsible for characteristics that affect color and quality of red wines. These include proanthocyanidins, flavanols, anthocyanins, phenolic acids, and stilbenes such as resveratrol. Red wine is one of the richest sources of polyphenols in the human diet. Moderate drinkers will consume polyphenols at levels well above the population average. These compounds are potent radical scavengers and antioxidants and have been associated with the prevention of age-related disorders such as cancers, neurodegenerative diseases, inflammation, and cardiovascular disease. The biochemical profiles of red wines change with aging. Grape pigments degrade and reform into new and more stable compounds over weeks, months, and years. We studied five single-vineyard red wines from vineyards in Napa, Sonoma, and Barolo that ranged from 20+ years-old to current barrel samples. Analysis was performed by an accredited wine laboratory (ETS Labs, Healdsburg, CA). Total monomeric and polymeric anthocyanins declined during aging. Malvidin-3-glycoside, catechin, and quercetin glycosides significantly declined. A notable trend toward decline was seen for caffeic acid, caftaric acid, and quercetin. Gallic acid, hydrolyzed from tannin over time, showed a nonsignificant increase with aging. Antioxidant capacity was unchanged by aging. Slow oxidation of red wine in the bottle leads to polymerization of phenolics. The phenolics are not lost, but combine to form new compounds. As these polymers are derived compounds, it is not clear what biologic activity they might have that is different from the “natural” compounds. Observed physiologic phenomenon may be affected by wine aging.

Funding Support: Desert Heart Foundation Wine and Heart Health Research Initiative, a private nonprofit research and education foundation; wine samples provided by participating wineries and private collections; lab analysis provided gratis by commercial lab

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**Impact of Red Blotch Disease on Cabernet Sauvignon and Chardonnay Grape Composition**

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Since 2011, when Grapevine Red Blotch Associated Virus (GRBaV) was identified as the causal agent of red blotch disease (RB), the disease has been found in several white and red winegrape growing regions in California. Vines with RB show symptoms like red veins on the leaf undersides and no rolling. In white varieties, RB symptoms include chlorotic regions within leaf blades. Currently, little is known about the impact of RB on grape composition. What is known is that in infected grapes, there is decreased sugar accumulation and delay in fruit maturation. Only anecdotal evidence exists regarding phenolics. The impact of RB on the chemical and phenolic composition of Cabernet Sauvignon and Chardonnay grapes, two important winegrape varieties, was investigated. During the 2014 and

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2015 harvests, Cabernet Sauvignon and Chardonnay berries were sampled from healthy and symptomatic, RB-infected grapevines. Samples were collected every two weeks from veraison to harvest from vineyards in Napa and Sonoma counties. Standard grape chemistries were determined during ripening and harvest (Brix, pH, titratable acidity, malic acid, tartaric acid, total anthocyanins, and tannins). Selected samples were also analyzed by RP-HPLC and phloroglucinolysis to determine the phenolic and tannin profiles of the grapes. Grapes harvested from RB-infected grapevines had less sugar accumulation (0 to 9% in Chardonnay and 4 to 20% in Cabernet Sauvignon), less total anthocyanins (0 to 16% in Cabernet Sauvignon), higher titratable acidity (0 to 3% in Cabernet Sauvignon and 11 to 16% in Chardonnay), and greater catechin concentration (46% in Cabernet Sauvignon) than noninfected vines.

*Funding Support: American Vineyard Foundation Science Without Borders (Brazil)*

#### Small-Molecule Fingerprinting and Carbohydrate Profile of Used Bourbon Barrels

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Bourbon whiskeys are typically aged between two and 23 years in newly charred White American Oak (*Quercus alba*) barrels. During whiskey maturation, the wood composition is transformed, resulting in new volatile compounds being added to the wood. The interaction of the freshly charred barrel with distillate, which contains compounds from the fermentation, results in volatile compounds that give bourbon its unique flavor. There is increasing interest in using used bourbon whiskey barrels to age other beverages such as wine and beer. Understanding the chemical and physical properties of barrels during aging may prove informative for producers wishing to enhance quality. To determine the volatile fingerprint in charred oak barrels during aging, we performed pyrolysis gas chromatography mass spectrometry (PYR-GC-MS) and high-performance liquid chromatographic analyses. Data illustrated numerous flavor notes that contribute to bourbon whiskey.

*Funding Support: NSF EPSCoR*

#### Differential Growth Effects of Native and Commercial Mycorrhizal Inoculants on Grapevine Rootstocks

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Grapevine roots associate with arbuscular mycorrhizal (AM) fungi, symbiotic organisms generally known for the array of benefits they provide host plants such as increased biomass, environmental stress tolerance, and pathogen protection. Although AM fungi likely play an important role in establishing young grapevines, the symbiosis needs to be better understood for AM fungi to be best utilized in grapevine management. For example, what AM fungi are best suited for inoculat-

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ing grapevines? Because commercial inoculants are produced using only one or a few AM fungal species grown on grass and forb roots in artificial conditions, it may be better to inoculate grapevines with more natural communities containing more diverse mixtures of AM fungi. This study compared the growth effects of natural and commercial AM fungal inoculants on grapevine rootstocks. We inoculated three rootstocks (Riparia gloire, 101-14 Mgt, and SO4) with natural AM communities (isolated from native woody shrubs located adjacent to vineyards) and commercial AM fungal strains. Rootstocks had greater biomass when inoculated with natural AM fungi than with the commercial isolates, both above and below the ground. Due to the nature of the natural inoculant, it is possible that the microbial community as a whole may be responsible for these growth differences, however this is a potential benefit to using whole soil or root communities compared to commercial additives.

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**Sensory Profiling and Chemical Analysis of Rosé Wines Elucidates Effects of Popular Production Techniques**

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Interest in and consumption of rosé wines has increased considerably worldwide in recent years. The allure of rosé is driven by its unique sensory profile and chemical composition, arrived at through certain vinification and viticultural practices. However, there is a lack of sensory descriptive analysis publications related to rosé. Therefore, insight into which production methods contribute to specific sensory qualities is needed. This study provides the first comprehensive interpretation of the relationships between the three most common rosé production techniques and their corresponding sensory properties. Two typical rosé cultivars, Syrah and Tempranillo, were used to make rosé wines using short maceration, saignée, or blending. Trained panelists used descriptive analysis and temporal dominance of sensation (TDS) to determine the sensory profiles of each wine. Effects of color perception on perceived attribute intensities were also explored. Chemical analysis was carried out on each wine and results were related to sensory data using multivariate analysis. There were clear sensory differences related to process method that could be related to pH and acidity differences in the wines.

*Funding Support: Ray Rossi Endowment*

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#### Genetic Diversity Analysis of Grapevine Rupestris Stem Pitting-associated Virus in Ontarian Vineyards

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Grapevine rupestris stem pitting-associated virus (GRSPaV; family *Betaflexiviridae*, genus *Foveavirus*) is a positive-sense, single-stranded RNA virus detected in grapevines worldwide. While definitive proof is still lacking, different strains of GRSPaV are associated with several grapevine diseases such as Rupestris Stem Pitting, Vein Necrosis, and Syrah Decline. Sequence variants of this virus form distinctive clusters and different clusters may correlate with different diseases. To improve understanding of the genetic diversity and pathological properties of GRSPaV, we analyzed the genetic diversity of viral sequence variants present in Ontarian vineyards. It was hypothesized that multiple sequence variants of GRSPaV are present in Ontarian vineyards and that variants of the –SY lineage, either alone or in combination with other agents, are responsible for the observed Syrah Decline syndrome. Using reverse-transcriptase polymerase chain reaction, genomic regions of the RNA-dependent RNA polymerase and the capsid protein were cloned, sequenced, and subjected to phylogenetic analysis. The vast majority of viral clones from table and juice varieties clustered with GRSPaV-SY, while some clustered with -VF1. In winegrape varieties, those with and without observable Decline symptoms had similar groups of variants overall. However, most clones derived from grapevines without observable symptoms clustered with -SY and -JF lineages. In symptomatic grapevines, the majority of clones clustered with the -SY and -VF1 groups, suggesting that these clusters of sequence variants may be involved with symptoms. A full-length cDNA clone corresponding to GRSPaV-SY is being constructed and its infectivity will be assayed in *Nicotiana benthamiana* and the natural host, grapevine. We hope that these studies will provide new insight into a potential correlation between certain viral variants and the Syrah Decline symptoms observed in Ontarian vineyards.

*Funding Support: NSERC Engage Grant, NSERC Discovery Grant*

#### Chemical Analysis of Wine with HS-SPME and GC-TOFMS for Target Screening and Non-Target Characterization and Comparison

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Chemical analysis of aromas associated with wine provides useful information for screening and understanding a product or process. Here, we use headspace solid phase microextraction (HS-SPME) as a sample preparation method to collect and concentrate volatile analytes in the headspace of a wine sample. The samples were then analyzed with gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS). This analytical technique offered untargeted and comprehensive chemical data for the samples that could also be probed for specific targeted compounds. A set of wine samples were spiked with 2,4,6-trichloroanisole at parts per trillion (ppt) to parts per billion (ppb) concentrations to simulate

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Enology and Viticulture – CONTINUED

the cork taint wine fault and to demonstrate the capability of this analytical approach to screen for and quantify this targeted analyte at concentrations near the sensory threshold. In addition to the targeted screening, the aroma analytes were characterized to gain insight into the overall aroma profile of the wine. Further untargeted comparisons between fresh and oxidized wine samples were made to determine specific chemical differences. By extending the analytical technique to two-dimensional gas chromatography (GC × GC), additional distinction between the samples could be determined from the increased peak capacity and lower limit of detection associated with GC × GC. These benefits provided the ability to detect more analytes within these complex samples and uncover additional chemical differences.

*Funding Support: LECO Corporation*

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**Characterization of Pierce's Disease Resistance in Germplasm Collected from the Southwestern United States and Mexico**

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The source of Pierce's disease (PD) resistance in the Walker lab breeding program is derived from a form of *Vitis arizonica* that Harold Olmo collected in Monterey, Mexico. This resistance is inherited as a single dominant gene named *PdR1* and is located on chromosome 14. To identify other forms of resistance that could be stacked to produce more durably and broadly resistant winegrapes, we evaluated over 250 accessions from the southwestern United States and Mexico. These accessions were greenhouse-screened for resistance and genetically characterized with chloroplast and SSR markers. Greenhouse screening identified **multiple** accessions that supported very low levels of PD's causal agent, *Xylella fastidiosa*. The genetic characterization identified five major groupings, within which 15 highly resistant accessions were grouped in clades different than those housing *PdR1*. Crosses were made among these resistant accessions and susceptible *V. vinifera* to develop small breeding populations that were greenhouse-screened to determine the mode of inheritance in the different resistant parents. To ensure the resistances differed from the *PdR1* locus, a limited mapping strategy was used to scan the chromosome 14 region. Twelve SSR markers that flank the *PdR1* locus and cover 3.5Mb of physical distance were used to genotype 704 seedling plants. Data from each population was analyzed to determine the distribution of resistance. Each marker was tested for deviation from normal segregation and haplotypes were constructed. The linkage data was used to carry out QTL analysis for each population. The resistant accessions will be discussed in terms of their genetic differences and potential to provide unique and strong sources of PD resistance for breeding.

*Funding Support: CDEA Pierce's Disease Board, and from the Louis P. Martini Endowed Chair in Viticulture, the Louise Rossi Endowed Chair in Viticulture, and support for Karla Huerta from CONAYCT, Mexico*

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#### Effects of Winery Wastewater on Soil, Grape Nutrition, and Wine Quality

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Many wineries are interested in recycling wastewater for irrigation. This project investigates the effects on winemaking when winery wastewater (WW) is recycled for irrigation. Water samples and soils samples were collected from one Napa Valley and one Sonoma vineyard. Leaf and berry samples were collected at veraison (mid-season) and at harvest, with harvested grapes being made into wine for a sensory trial. All samples were analyzed for Na, Mg, K, and Ca metals by inductively coupled plasma mass spectrometry (ICP-MS), and the grape and wine samples were also analyzed for total phenolics and tannins. The presence of grape compounds and detergents caused the WW concentrations of K to be 143x higher than the control irrigation water. The soil samples showed significant accumulations of Na and K between treatments while the leaf samples showed significant differences in Na, Mg, K, and Ca between treatments, but the majority of these were slight. The grape samples showed significant differences in Na and Ca concentrations while the wine showed minor but significant differences in all metal concentrations. The sensory study on the Napa vineyard showed no statistical significance between the finished wines.

*Funding Support: Agricultural & Natural Resource Competitive Grants*

#### A Mechanistic Model for Vegetative Vigor in Grapevine

**Ines Hugalde,\*** Cecilia Aguero, Nina Romero, Felipe Barrios-Misas, Andy Nguyen, Andrew Walker, Andrew McElrone, and Herman Vila

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This study describes the construction of a theoretical model for plant vigor. The model conceives vigor as the result of the interaction of external environmental stimuli, mainly soil and atmospheric conditions, and plant physiological behavior. Soil water status directly affects root hydraulics. Atmospheric humidity, water vapor pressure deficit, light, and temperature affect leaf water potential and stomatal conductance. These responses to external inputs influence plant gas exchange (stomatal conductance and photosynthesis), tissue turgor, and cell expansion. These factors, also influenced by hormonal signals, determine total C assimilation and biomass partitioning, affecting plant growth and vigor. To parameterize and test our model, we studied the F1 progeny from a cross between Ramsey and Riparia Gloire de Montpellier, rootstocks that confer high and low vigor, respectively. We evaluated 138 genotypes, three replicates each, for 60 days in a greenhouse at UC Davis, California, in summer 2014 and 2015. Each plant was pruned to a single shoot and watered daily. In 2014, after day 45, 50 genotypes were subjected to a 50% water deficit, based on initial weight at full capacity. Shoot growth rate, leaf area, and dry biomass were measured for the complete population (including the stressed subset), while hydraulic conductance, stomatal conductance, water po-

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tential, and chlorophyll content were measured in the 50 genotypes. In 2015, we repeated the evaluation, adding photosynthesis and root hydraulic conductance measurements in the own-rooted population and a grafted subset, using Cabernet Sauvignon as scion. We will present a preliminary model showing the main mechanisms, inputs, and outputs that control plant vigor. Future goals include completing the model, running simulations, and validation.

*Funding Support: Estación Experimental Agropecuaria Mendoza, INTA*

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**Physiological and Genetic Control of Vigor in a Ramsey x Riparia Gloire de Montpellier Population**

**Ines Hugalde\***, Summaira Riaz, Cecilia Agüero, Nina Romero, Felipe Barrios-Masias,

Andy Nguyen, Hernan Vila, Andrew McElrone, and Andrew Walker

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Rootstocks are a valuable tool for viticulturists, conferring numerous advantages, among which vigor control is important. This research studied F1 progeny from a cross between Ramsey and Riparia Gloire de Montpellier, rootstocks that confer high and low vigor, respectively. We hypothesized that vigor, defined as canopy biomass, correlates with growth rate, leaf area (LA), specific leaf area (SLA), biomass partitioning, and plant and root hydraulic conductance and also that these variables could be associated with genetic markers. We evaluated 138 seedlings from this cross, three replicates each, for 60 days in a greenhouse at UC Davis, California, during summer 2014 and 2015. Each plant was pruned to a single shoot and watered daily. Shoot growth rate, leaf area, and dry biomass were measured in the complete population both years. In 2014, a subset of 50 genotypes was subjected to water deficit (50% of soil water content) after day 45 and evaluated for plant hydraulic conductance, root hydraulic conductance, stomatal conductance, and water potential. In 2015, a subset of 50 genotypes, own-rooted or grafted with Cabernet Sauvignon, was evaluated for photosynthesis and root hydraulic conductance. The 2014 data showed significant QTLs for LA, SLA, and partitioning indices on chromosomes 1, 4, 16, and 5: accounting for 20% of LA and 10 to 14% of SLA, and partitioning indices explained variation. QTLs for LA have also been found on chromosome 4 in Ugni blanc. Hydraulic data from 2014 and mapping data from 2015 are being analyzed.

*Funding Support: Estación Experimental Agropecuaria Mendoza, INTA*

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**Does Production of High Levels of Sulfite Correlate with Undesirable Sulfide Formation in Commercial Wine Yeasts?**

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While regulations for making organic wine in the United States prohibit addition of sulfite during processing, they do not disallow the presence of sulfite. Certain wine strains of *Saccharomyces cerevisiae* produce relatively high levels of sulfite (>10

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ppm). This production is a result of natural metabolic processes that yeast share with other microbes and plants; specifically, synthesis of the sulfur-containing amino acids methionine and cysteine. We are evaluating the factors that control high-sulfite production to determine strain suitability for production of organic white wines that do not undergo malolactic fermentation or aging, e.g., Pinot gris. The relationship between high-sulfite production and high sulfide formation in these strains has not been examined systematically under vinification conditions. Initially, we assessed commercial wine strains for both sulfite and sulfide production. A handful of high-sulfite producers were selected for further analysis. Spores of the high sulfite-producing strains were crossed to a low sulfite-producing laboratory strain and are currently being subjected to genetic analysis. Specifically, a rapid, high-throughput sulfite assay suitable for microtiter plate analysis was developed to follow this trait among hybrid progeny. A diagnostic medium is being used to follow hydrogen sulfide production. Two strains containing the previously characterized low-sulfide *MET10-932* allele were found to produce high levels of sulfite and were confirmed to produce no sulfide. In contrast, another strain co-produced high levels of both sulfite and sulfide. These findings indicate that the relationship between high-sulfite production and undesirable high-sulfide formation varies among strains.

*Funding Support: USDA-ARS*

### Effects of Must pH on Red Grape Skin Tannin Extraction

**Eri Inoue**, Haruka Kobayashi, Guangxian Liang, Fumie Saito, Masashi Hisamoto, and Tohru Okuda\*

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Polyphenols are important compounds in red wine, and mechanisms for their extraction have been studied intensively. Especially in red wine, grape skin polyphenols are vital contributors to taste and color. However, skin tannin rapidly decreases in red grapes such as Muscat Bailey A (Muscat Hamburg × Bailey, MBA) during must fermentation. In this study, we focused on the adsorption of tannin to insoluble polysaccharides from grape skin and carried out experiments on model wine solutions (12% ethanol and 5 g/L potassium bitartrate) at pH 2.5 to pH 5.0. At pH 3.5, ~30% of skin tannin adsorbed to insoluble polysaccharides from grape skin. High (70%) and low (30%) affinities with skin tannin were noted at certain pH values (pH 4.2 and pH 3.2). We also examined must pH that affects MBA skin tannin extraction in model experiments. MBA skin tannin was extracted into model wine at pH 3.2, 3.7, or 4.2. Bovine serum albumin (BSA) binding tannin concentrations were analyzed (protein precipitation method). At pH 3.2, double the skin tannin was extracted than at pH 4.2. The results suggest that must pH in fermentation is important for skin tannin extraction into red wine.

*Funding Support: Grant-in Aid for Scientific Research*

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**Influence of Direct Root Zone Microirrigation on Production of Cabernet Sauvignon in the Pacific Northwest**

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Vines receiving drip irrigation delivered directly into the lower root zone yielded 70% of commercial production while receiving only 15% as much water as vines that received full commercial rates of surface drip irrigation during 2015, the hottest and driest year on record in south-central Washington. Preliminary data supports our hypothesis that water use efficiency can be significantly improved through deep sub-surface irrigation. Vines receiving direct root zone irrigation at rates reduced to 60, 30, or 15% of commercial drip irrigation produced individual clusters with more, but smaller, berries than did clusters from vines receiving full rates of surface drip irrigation. Preliminary findings from a replicated, randomized complete block experimental design conducted in a commercial vineyard in Washington's Red Mountain AVA suggest that this form of subsurface microirrigation has the potential to conserve at least half of the water required for quality grape production under contemporary surface drip irrigation. Additionally, we hypothesize that this form of microirrigation can produce grapes of more desirable quality for producing premium red wines. Grape production was increasingly higher with depth and amount of applied water, yielding 90% of commercial production in plots receiving 60% of the commercial irrigation rate delivered at slightly less than a meter below the soil surface. Interrupted irrigation delivery provided no advantage over uninterrupted delivery during 2015; however, this could have been influenced by the soil texture at this site. Vines receiving direct root zone irrigation at reduced volumes of total water showed more plant water stress than those receiving full commercial rates of irrigation via surface drip irrigation, based on samples of midday stem xylem pressure potential. This experiment will be expanded to other sites and grape varieties in 2016.

*Funding Support: Washington Wine Commission Wine Advisory Committee, NW Center for Small Fruit Research, Washington State Dept. of Agriculture Specialty Crop Block Grant Program*

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**A Model for Predicting Anthocyanin Extractability in Red Wines**

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The relationship between grape and wine phenolics is of key interest for the wine industry to predict wine quality from analysis of grapes. Research suggests that the physical properties of skin tissue are related to the composition and extractability of compounds with importance to wine quality. What is lacking is an understanding of the relationship between grape texture, extractability of grape components, and wine quality under real winemaking conditions. Although analytical texture methodologies are in place, routine analytical procedures to predict wine quality

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are still unavailable. To make such assessment more applicable in a winery setting, prediction of the anthocyanin composition and color of red wines from the detailed anthocyanin composition and skin texture was investigated using a multiple regression model. Grape extracts and wines were produced from two *Vitis vinifera* L. varieties, Merlot and Cabernet Sauvignon, grown in different regions in California, from warm interior valley sites to cool north coast sites. A linear combination of three independent variables could accurately predict the total concentration of anthocyanins in wines. Total skin anthocyanins, skin hardness, and skin weight were significant predictors of wine color, and the model developed was independent of region and cultivar. With this model, winemakers could predict anthocyanin extractability and adapt the process accordingly.

*Funding Support: The California State University Agricultural Research Institute (ARI), E & J Gallo Winery*

### Using Leaf Excision during Gas Exchange Measurements to Increase Sample Size

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For many research projects, gathering leaf gas exchange data is essential. However, due to the length of time required to make measurements, the number of leaf samples gathered is often limited. To increase sample size, our research compared gas exchange of excised grapevine leaves to gas exchange of leaves remaining on the vine. Vines of *Vitis vinifera* Grenache, Cabernet Sauvignon, Chardonnay, Tempranillo, and Cabernet franc (all grafted onto 110R rootstocks) were sampled during 2013 and 2014. Research was conducted in an established vineyard near Lubbock, Texas. Using auto program mode, two LI-6400 XT machines simultaneously measured gas exchange on nearby leaves from the same shoot on the same vine. Measurements were recorded every 30 sec. After 120 sec, one leaf was excised and the auto program continued an additional eight minutes. Gas exchange means for excised and nonexcised leaves were graphed over time. In addition, gas exchange means at 60, 90, 120, 150, and 180 sec after the auto program began were exposed to ANOVA, and means were separated by Fisher's least significance difference procedure. Data indicate gas exchange for nonexcised leaves of all varieties remained stable during the measurement period. In contrast, gas exchange for excised leaves of each variety changed during the measurement period. However, there was a period of time during which excised leaf gas exchange was not different from gas exchange measured prior to leaf excision. Therefore, it appears leaf excision might be one tool researchers could use to increase sample size when collecting vineyard leaf gas exchange data.

*Funding Support: Texas AgriLife Research and Extension*

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**Identification of Bacterial Metabolite Inducer(s) of the [Gar<sup>+</sup>] Prion of *Saccharomyces cerevisiae***

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In response to environmental stress, *Saccharomyces cerevisiae* can induce prion states within the yeast population that behave in an adaptive and heritable manner. Prions allow a portion of the population to sample new survival strategies without affecting long-term fitness. Induction of the [GAR<sup>+</sup>] prion bypasses glucose repression, resulting in reduced fermentative capacity. Certain strains of bacteria excrete small molecule effector(s) that act as the induction signal for establishment of [GAR<sup>+</sup>]. In particular, wine spoilage lactic and acetic acid bacteria are strong inducers of the [GAR<sup>+</sup>] prion. Thus, one explanation for the loss of fermentative activity by wine yeast in the presence of bacteria is induction of the prion state. To understand the mechanism of induction, identification of the bacterial inducer is necessary. The inducer is diffusible and therefore present in the medium following bacterial growth. To identify bacterial metabolite(s) that act as the inducer signal for the induction of [GAR<sup>+</sup>], wine spoilage bacteria from the UC Davis Culture Collection were screened for prion induction under a variety of growth conditions. Bacteria were grown in media of varying composition, the medium was filtered to remove bacterial cells, and yeast was incubated in the spent medium for varying amounts of time and then tested for the induction of the prion. Yeasts suspended in spent medium from *Acetobacter pasteurianus*, grown on glycerol and glucosamine, demonstrated induction of the [GAR<sup>+</sup>] phenotype. Growth in other media did not induce the prion, suggesting that either the inducer was not synthesized or that the yeast were protected against induction by other components in these media. This analysis provided useful conditions in which to assess the composition of the spent medium or exometabolome. The findings from the exometabolome analyses will be discussed.

*Funding Support: American Vineyard Foundation*

**Pruning Systems and Applied Water Interact on Productivity and Phenolic Composition of Zinfandel**

**Clinton Nelson**, James Kennedy, and S. Kaan Kurtural\*

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A trial was conducted in the hot climate of central California to assess the interactive effects of pruning systems and deficit irrigation methods on canopy architecture, yield, water productivity, and phenolic composition of Zinfandel under exceptional drought for three years. The pruning systems were cane-pruned (CP, six canes with eight nodes), spur-pruned (SP, 22 two-node spurs), and mechanically-pruned (MP, 100 mm hedge). The deficit irrigation treatments were sustained deficit irrigation (SDI) at 80% of crop evapotranspiration (ET<sub>c</sub>) from budbreak to leaf fall and regulated deficit irrigation (RDI) at 80, 50, and 80% of ET<sub>c</sub> between budbreak and fruit set, fruit set to veraison, and veraison to leaf fall, respectively. The research vineyard received 87, 30, and 27% of the 10-year aver-

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age winter precipitation in years 1, 2, and 3 of the study. In year 1, the combination of CP and RDI was recommended based on reproductive and reduced water footprint. In years 2 and 3, the combination of MP and SDI was recommended based on sustainable yield, optimum leaf area to fruit ratio, and greater flavonol, flavan-3-ol monomers, and anthocyanin concentrations in berry skin. Recommended leaf area to fruit ratio and Ravaz index productivity indices were adjusted based on results for this hot climate region to reach commercial maturity for the cultivar studied. The study provides information to help growers identify combinations of pruning systems and deficit irrigation methods that may optimize reproductive, vegetative, and water productivity while accumulating commercially acceptable flavonol, flavan-3-ol monomers, and anthocyanin concentrations in berry skin in hot climates under exceptional drought.

*Funding Support: American Vineyard Foundation*

#### How Flavonoids Stimulate Browning

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Wine oxidation is a chemical process that changes the chemical and sensory profile of wines. A degree of oxidation is beneficial for red wine to enhance color and to reduce astringency, but overoxidation results in negative characteristics. In wine oxidation, phenolics are oxidized to quinones and these reactive compounds have a key impact on the outcome, degrading color and flavor. Antioxidants can prevent degradation by nucleophilic reactions with or reduction of the quinones. The A-ring on flavonoids is an important nucleophile and should arrest oxidation by quenching the quinone. However, when we attempted to quantify that reaction rate, we observed that electron transfer occurred more quickly between the quinone and B-ring of flavonoid, resulting mostly in the flavonoid quinone, extending rather than arresting the oxidation reaction. The flavonoid quinone is known as a precursor of browning products. The fact that the electron transfer reaction was faster than the nucleophilic reaction helps explain why flavonoids in wine can stimulate browning rather than arresting oxidation.

*Funding Support: American Vineyard Foundation*

#### Comparative Response of Six Grapevine Rootstocks to Inoculation with Arbuscular Mycorrhizal Fungi (*Rhizophagus irregularis*)

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Grapevines benefit from root system colonization by arbuscular mycorrhizal (AM) fungi (Glomeromycota) through enhanced nutrient uptake, increased resistance to soil pathogens, and improved tolerance to abiotic stresses like drought and salinity. However, the response of young grapevines to inoculation with AM fungi varies with rootstock variety, and the underlying mechanisms involved in this variation

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are unknown. We analyzed the root systems of young greenhouse-grown plants of six rootstocks (3309 Couderc, Riparia Gloire, Ramsey, 101-14 Millardet et de Grasset, Swarzmman, and Teleki 5C) with and without colonization with AM fungi (*Rhizophagus irregularis*) to characterize their morphological and architectural responses to AM infection. The response of vegetative growth, specific leaf area, and other physiological traits of the vines such as leaf P and N to AM infection was also evaluated. Over 20 weeks postinoculation, vine growth in two rootstocks was enhanced by AM infection. Root system growth and architecture were also altered by AM infection, and these effects also varied among rootstocks. The results of this study will help to elucidate how interactions between grapevine rootstocks and AM fungi may be beneficial to establishing new vineyards.

*Funding Support: Agriculture & Agri-Food Canada BC Wine Grape Council*

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**Effect of Potassium and Ethepon on Color and Anthocyanin Concentrations in Berries of Red Globe (*Vitis vinifera* L.)**

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In red cultivars like Red Globe, coverage of color on the berries is a critical quality variable. In the province of San Juan, Argentina, environmental conditions favor high concentrations of soluble solids and low concentrations of tartaric acid. Such conditions also prevent good color development on the berries without applications of synthetic growth regulators like ethephon. Currently, countries that import table grapes are demanding reduced use of ethylene, which may constitute a barrier-tariff in the future. This context motivated field trials to evaluate the effect of potassium on color and anthocyanins in berries of Red Globe. The trials were carried out during two seasons. During 2013-2014, the treatments were TA: control; TB: 700 cc 48% ethephon; TC: 700 cc 48% ethephon + 3 kg Color T; TD: 3 kg color T; and TE: 6 kg color T. During 2014-2015, the treatments were: TA: control; TB: 500 cc 48% ethephon; TC: 700 cc 48% ethephon; TD: 500 cc 48% ethephon + 3 kg color T; TE: 500 cc 48% ethephon + 3.6 kg Raisan K; TF: 6 kg color T; and TG: 7.2 kg Raisan K. During both seasons, the treatments were made on clusters with 50% veraison. For TD and TE in season 2013-2014 and TF and TG in 2014-2015, half of the doses were applied at the mentioned time and the rest was applied one week later. The color T product employed consisted of 46% N, 30% K, and 1.5% Mg while Raisan K was 24% N and 2.5% chitosan. The anthocyanin concentration and color of berries in 2013-2014 was greatest in TC clusters, followed by TE. In 2014-2015, the TD and TE treatments had the most anthocyanin and berry color.

\*indicates corresponding author

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#### **Sulfur Dioxide Addition Alters *Saccharomyces cerevisiae* Populations and Wine Sensory Characteristics in Spontaneous Fermentations**

**Sydney Morgan,\*** Chrystal Scholl, Natasha Benson, Morgan Stone, and Daniel Durall

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Sulfur dioxide is added before the onset of alcoholic fermentation at most commercial wineries to prevent growth of spoilage microorganisms and help create an environment that promotes rapid colonization of the grape must by *Saccharomyces cerevisiae*. *S. cerevisiae* is a winery-resident yeast and is rarely found on healthy grapes in the vineyard. It performs the bulk of alcoholic fermentation and is a major contributor to the final aroma and flavor of wine. As such, it is extremely important to understand how the winemaker's addition of sulfur dioxide at crush can indirectly impact the strains of *S. cerevisiae* conducting alcoholic fermentation. This study investigates the effects that four different levels of initial sulfur dioxide addition (0, 20, 40, or 80 mg/L SO<sub>2</sub>) have on the *S. cerevisiae* strains present during spontaneous fermentations of two grape varieties at two Okanagan wineries. Yeast isolates collected from samples taken throughout fermentation were identified at the strain level using microsatellite analysis. *S. cerevisiae* strain composition was significantly impacted by the level of SO<sub>2</sub> added at crush. Each SO<sub>2</sub> treatment had a significantly different strain composition at each winery, and each winery had a completely unique strain composition. The dominant strains throughout fermentation (those comprising at least 10% of the relative strain abundance) were commercial strains at both wineries, with the exception of one strain at one winery, which was of unknown, assumed indigenous, origin. Sensory analysis was conducted at one winery and sensory differences were found between treatments. The results of this study highlight the dominance of commercial strains in commercial winery environments and demonstrate that initial sulfur dioxide addition indirectly alters the *S. cerevisiae* populations conducting spontaneous fermentations, resulting in altered sensory characteristics.

*Funding Support: National Sciences and Engineering Research Council of Canada (NSERC), British Columbia Wine Grape Council (BCWGC)*

#### **Grapevine Shoot and Cluster Development as a Function of Arm Positioning along the Cordon**

**Brooke Robertson,** Courtney Gillespie, M. Anderson, M. Andrew Walker, and Jean Dodson Peterson\*

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Cordon-trained, spur-pruned vines often experience developmental delays at the mid-cordon arm positions. The delay in budbreak, shoot growth, and cluster development often translates into significant variation in the timing of cluster ripeness near harvest. The main objective of this research is to determine the role of bud number and pruning technique in minimizing developmental variation at the head-, mid-, and end-cordon positions of a vine. This study was conducted in a Cabernet Sauvignon vineyard in Oakville, California, on bilateral cordon-trained, spur-pruned vines on 6 x 8 foot spacing. Vines were pruned to either

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one- or two-bud spurs, for a total of 12 or 24 buds per vine, respectively. Light interception at the fruiting zone was measured via an Accupar LP-80 photosynthetic radiation sensor and then normalized by removing leaves in the denser, two-bud spur treatments. In addition to shoot length, diameter, percent berry set, berry maturity indices, spur diameter, and pruning weights, the timing of key phenological stages was tracked. Significant variation was found among shoot lengths of each treatment and among positions along the cordon. Middle positions had significantly less shoot growth than either the head or end positions, and one-bud pruning gave longer shoot growth than two-bud pruning. These study results will be used to make pruning level and vine training recommendations that will balance productivity across the vine, regardless of arm positioning.

*Funding Support: California State University Agricultural Research Institute*

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**Effects of Hyperoxidation and Storage Temperature on the Flavor Profile and Sensory Quality of Riesling Wine**

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Exposure to oxygen during white wine production is generally considered to negatively impact color, aroma, flavor, and shelf life. However, hyperoxidation, the intentional exposure of recently pressed juice to high levels of oxygen, has been used to initiate enzymatically-controlled oxidation cascades that remove the phenolic precursors of oxidizable compounds. Removal of these precursors prior to vinification may lead to a product with improved color over time, greater shelf stability, and less harsh or bitter flavors. Optimized storage temperatures can also increase the shelf life of white wines by maintaining terpene and ester contents and by preventing the formation of new detrimental flavor constituents. The overall effects of hyperoxidation and storage conditions on white wine quality is still in dispute and has created a need to critically evaluate the combined effects of hyperoxidation and storage temperatures on Riesling wines. This study examines control and hyperoxidated wines at three storage temperatures (63, 75, and 90°F) through chemical and sensory evaluations over time. Initially, a trained sensory panel detected no significant differences in aroma or flavor characteristics between hyperoxidated and control wines before entering storage treatments. After one year of storage, wines held at 63°F retained significantly higher sensory ratings for overall aroma intensity, fruit aroma, and fruit flavor than wines stored at the higher temperatures. Wines stored at 90°F developed darker colors, oxidized aroma characteristics, and a loss of varietal flavor attributes. These detrimental characteristics were first detected in the control wines before they were observed in the hyperoxidated wines. The sensory results of this study were compared with SPME-GC-MS analyses of the aromatic volatile compounds. The successful determination of optimized hyperoxidation treatments could potentially benefit both wineries and consumers with longer-lasting white wines even under challenging storage conditions.

*Funding Support: Ohio Grape Industries Committee and Ohio State University*

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### Enology and Viticulture – CONTINUED

#### Do Online Disease Management Courses Change Grower Behavior? Powdery Mildew and California Grapegrowers

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Over 200 online courses are licensed by the California Department of Pesticide Regulation (DPR) and available to growers in California, but little is known about the extent to which these courses affect grower behavior. This paper explores the effect of an online training course on pesticide application behavior of grapegrowers and the resulting change in disease management costs and environmental impact. The management practices examined in this paper are pesticide applications by grapegrowers in California to prevent and treat powdery mildew. We combine data on growers who completed the online course titled “Grape Powdery Mildew Control in California Vineyards” between 2004 and 2011 with Pesticide Use Reports (PUR), disease pressure forecasting data, and Pesticide Use Risk Evaluation (PURE) indicators to assess the impact of the course on pesticide applications made by grapegrowers to control powdery mildew. We find that the growers adjust their pesticide applications according to the major guidelines of the online course, but there was significant heterogeneity in response among different categories of grapegrowers. As a result, growers in three of the four major grapegrowing regions in our sample experienced higher powdery mildew management costs after completing the course: between \$14 and \$84 per acre annually. We also found increased environmental impact of powdery mildew management post-class. We conclude that online courses are potentially an important tool in guiding grower behavior, but the course content and targeting of the audience are critical to achieve a specific behavioral response.

*Funding Support: Giannini Foundation of Agricultural Economics, University of California; VitisGen Project: Accelerated grape cultivar improvement via phenotyping centers and next generation markers. SCRI USDA Competitive Grant Award No: 011-51181-30635*

#### Elucidating Contributions of Vineyard Site to Phenolic Profiles of Pinot noir Wines

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Correlations between vineyard site and wine have historically been limited due to lack of uniformity in scion and rootstock clone and lack of controlled pilot-scale winemaking conditions, particularly temperature. Our work aims to minimize these sources of variation by using a single combination of scion and rootstock. In addition, we maintained highly controlled fermentation conditions by using automated 200-L fermentation vessels at the UC Davis Teaching and Research Winery. Clusters were hand-harvested from 10 vineyards comprising the same combination of scion, Pinot noir clone 667, and rootstock 101-14 Mgt. The vineyards were planted at locations spanning a distance of more than 650 km and

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Enology and Viticulture – CONTINUED

represented the Santa Maria Valley, Arroyo Seco, Carneros, Sonoma Coast, Russian River Valley, and Mendocino American Viticultural Areas (AVAs). The fruit was destemmed into fermentation vessels and inoculated with a strain of *Saccharomyces cerevisiae*. These vessels offer a high degree of automated temperature control, facilitating uniform fermentations. After primary fermentation, wines were inoculated with a strain of malolactic bacteria. Upon completion of MLF, wines were sampled for analytical characterization. Data characterizing wine phenolics were obtained using high-performance liquid chromatography (HPLC). The data were analyzed using analysis of variance (ANOVA) measuring for the effects of vineyard. Approximately 20 phenolic compounds were identified that significantly differentiated the wines. The compounds included hydroxycinnamic acids, flavan-3-ols, flavonols, and anthocyanins. Principal component analysis (PCA) was used to characterize vineyards using only significant compounds. By minimizing variation in scion/rootstock combination and in fermentation, AVAs could be separated by their phenolic compound profile; however, some vineyards within an AVA had dramatically different profiles. These results suggest that factors such as unique microclimates or soil conditions may have an effect. These details will be explored in future work as will the consistency of phenolic compounds from these sites in subsequent vintages.

*Funding Support: Jackson Family Wines*

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**Impacts of Cluster Thinning and Cluster-zone Leaf Removal on the Hormone Dynamics of Ripening Pinot noir Berries**

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Two common, yet expensive, practices used in viticulture are cluster thinning and cluster-zone leaf removal, which are intended to alter environmental conditions in hopes of causing the vine to respond in a favorable manner (e.g., improved fruit quality or disease management). Because the vine's response to environmental changes is principally hormone-mediated, we profiled the active forms, conjugates, and precursors of auxin, cytokinin, gibberellin, abscisic acid, brassinosteroid, jasmonic acid, and salicylic acid to understand the physiological effects of cluster thinning and leaf removal on the dynamic accumulation of these compounds in the berry. Similarly-developing berries were identified during the growing season and their tissues separated (seed, pulp, and skin). For the cluster-thinned treatment, clusters were thinned to 0.5 clusters/shoot; 100% of the cluster-zone leaves were removed for the leaf-removal treatment. Phytohormones were extracted using a targeted method developed within our laboratory. Preliminary findings from the first year's data are consistent with literature, but revealed a clear tissue- and stage-specific distribution of the bioactive forms of hormones. We also observed new accumulation patterns for gibberellic acids and brassinosteroids during preripening stages in skin and pulp, suggesting a role in ripening initiation. The treatment effects were largely negligible for many hormones. However, treatment effects reduced hormone concentrations in several cases. Leaf-removal treatment effects included a preveraison decline in abscisic acid, and both treatments

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reduced concentrations of indole-3-acetic acid (auxin), trans-zeatin (cytokinin), and castasterone (brassinosteroid) in both pericarp tissues. Though both treatments reduced hormone concentrations, leaf-removal treatment effects were more significant than those of cluster-thinning. The implications of these early findings are yet opaque but are likely to be clarified with a second year of data.

*Funding Support: Oregon Wine Board*

### Influence of Juice Turbidity, Hyperoxidation, and Skin Contact on Chardonnay Wine Mouthfeel

**Anthony Sereni**,\* Elizabeth Tomasino, and James Osborne

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This study investigated the impact of various prefermentation treatments on sensory characteristics of Chardonnay wine, with a focus on mouthfeel. Chardonnay grapes were harvested from Oregon State University's vineyard in September 2015. After destemming and pressing, the juice was subjected to various treatments. A portion of the juice was cold-settled for various lengths of time to produce juices with high (>700 NTU) to low (250 NTU) turbidity. Another portion of juice was settled overnight (250 NTU) and then hyperoxidized by bubbling oxygen into the juice until the dissolved oxygen measured >15 ppm (95% saturated). In addition, a portion of grapes were not immediately pressed after destemming but were soaked on the skins for 2 hr before pressing (skin contact treatment). After settling overnight, a portion of this juice was hyperoxidized (skin contact + hyper-ox). Three L fermentations of each treatment were performed in triplicate. Treatments were inoculated with *Saccharomyces cerevisiae* (D47) and placed in a temperature-controlled room at 15°C. After fermentations were complete, the wines were inoculated for malolactic fermentation by addition of *Oenococcus oeni* (Beta). At the completion of MLF, wines received a 50 mg/L SO<sub>2</sub> addition, were cold-settled, racked, sterile filtered, and bottled. Samples were taken and frozen at -80°C for volatile analysis. Absorbance at 280 nm (total phenolics) and 320 nm (hydroxycinnamic acids) differed among treatments. Wines that underwent hyperoxidation contained the least total phenolics while skin contact wines had the most. Hyperoxidation following skin contact reduced total phenolics. Whether these treatments modified the mouthfeel of the wines will be assessed using a winemaker sensory panel with results being analyzed by citation frequency technique.

*Funding Support: Oregon Wine Board*

### Revisiting an Old Problem: Flower Necrosis in Grapevines

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A series of experiments was carried out on Pinot noir grapevines to better understand flower necrosis. Previous research on late bunch stem necrosis indicated that accumulation of putrescine was the causal agent. It has been assumed that putrescine also causes flower necrosis, but this has not been confirmed. We tested

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whether putrescine accumulation causes flower necrosis by supplying various metabolites, including agmatine, to single-node flower cluster cuttings or by applying metabolites to developing clusters via a needle-delivery method in the field. Both approaches showed that high levels of putrescine in the rachis can cause flower necrosis and induce pedicel abscission in the field. The concentration of putrescine that induced flower necrosis in the field was similar to previous work on late bunch stem necrosis. However, further work comparing healthy and necrotic clusters from Pinot noir grapevines grown in sand-culture (with a history of flower necrosis) showed that flower necrosis was not due to accumulation of putrescine. These findings, combined with other field observations, led to the hypothesis that flower necrosis may also be caused by an imbalance in the root to shoot ratio of vines. This hypothesis was tested by manipulating shoot number in the sand-culture vines and in other vines with no prior history of flower necrosis. In both, flower necrosis correlated with lower shoot density per vine. In addition, root pruning of vines partially reversed the impact of low shoot density on flower necrosis. These findings show that while putrescine can induce flower necrosis in artificial experiments, another, presently unknown mechanism related to coordinated root and shoot development (and/or transport and sensing) causes flower necrosis in the field. Vineyard blocks with a history of flower necrosis could be improved by increasing shoot density or limiting root development.

*Funding Support: USDA-ARS*

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**Volatile Sulfur Compounds in Pinot noir Wine Postfermentation: Role of Nitrogen Composition and Elemental Sulfur**

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This study examined the impact of interactions among nitrogen concentration, composition, and elemental sulfur ( $S^0$ ) on volatile sulfur compound (VSC) content of Pinot noir wine. Fermentations were conducted using Pinot noir grapes where yeast assimilable nitrogen (YAN) was adjusted from 110 mg N/L to 250 mg N/L with either diammonium phosphate (DAP) or an amino acid mixture matching a typical Pinot noir grape composition. Ten mg/g  $S^0$  was also added to some treatments. DAP additions increased production of  $H_2S$  by *Saccharomyces cerevisiae* UCD522 while amino acid additions decreased  $H_2S$  formation regardless of  $S^0$  addition. Fermentations with both DAP and  $S^0$  produced the most total  $H_2S$ , with 35 to 45% more  $H_2S$  than ferments where only  $S^0$  or DAP additions were made. YAN concentration and composition and  $S^0$  also impacted the concentration of other VSCs in the wine postfermentation. In particular, the addition of  $S^0$  increased the concentration of methyl thioacetate (MeSOAc) in the wines as did higher YAN concentrations. The type of nitrogen added (ammonium vs. amino) had less impact on MeSOAc concentration than the increase in YAN did. This suggests that while the presence of  $S^0$  is one factor impacting the concentration of VSCs postfermentation, high YAN concentrations may also play a role. Experiments were also conducted to reduce the amount of residual  $S^0$  on Pinot noir grapes prior to fermentation. Pinot noir grapes received an application of  $S^0$  (wettable or microthiol) one week prior to harvest. Prefermentation cold soaking the grapes followed by draining off the juice

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reduced the amount of  $S^0$  remaining on the grapes and resulted in significantly less  $H_2S$  production during fermentation. Cold settling the juice that was drained off decreased the concentration of  $S^0$  below detection and allowed the juice to be added back to the original grapes.

*Funding Support: Oregon Wine Board*

### Evidence of Phenological Shoot Autonomy in Grapevines

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The cluster-thinning practice called green thinning is commonly performed to improve uniformity of ripeness. It is based upon an underlying assumption that shoots on the same grapevine are phenologically autonomous. There is no empirical evidence to support this assumption. The objective of this study was to test this assumption. A field trial was established comparing early pruning (EP) versus late pruning (LP) of spurs on separate vines (SV), opposite cordons on the same vine (OC), and alternate spurs on the same vine (AS). Phenological stages were followed on shoots from one vine per replicate for each treatment combination using an updated Eichhorn-Lorenz method from budbreak to the end of veraison. There was a significant difference in median phenological stage between EP and LP spurs for all vine-level treatments throughout the year. Median bloom dates and median veraison dates were delayed to the same degree by late pruning applied either to separate vines (SV), opposite cordons (OC), or alternate spurs (AS). Bloom was delayed 15 days with late pruning and veraison was delayed six days regardless of whether or not it was done on separate vines or the same vine. There was no significant interaction between pruning date and vine treatment on phenology throughout the season, which supports the hypothesis of shoot autonomy. Fruit maturity sampling after veraison and at maturity also showed a significantly lower Brix in LP vines for all vine treatments and no interaction between pruning date and vine level. There was, however, a significant interaction of pruning and vine level on pH at maturity. The results provide evidence for phenological shoot autonomy in grapevines.

*Funding Support: Treasury Wine Estates*

### Influence of Lysozyme Addition on Hydroxycinnamic Acids and Volatile Phenols during Yeast and Bacteria Cofermentation

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Most yeast and bacteria species in wine can metabolize hydroxycinnamic acids under winemaking conditions to form volatile phenols by decarboxylation. During fermentation, the release depends on yeast strain, the availability of free hydroxycinnamic acids, and the concentration of phenolic inhibitors. Our studies performed with Chardonnay and Pinot blanc fermentations from two vintages suggested that lysozyme addition to the juice prior to fermentation does not only affect bacterial activity, but also the release of hydroxycinnamic acids from their

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tartrate esters. This increases the theoretical potential for volatile phenol formation because microorganisms can only metabolize free hydroxycinnamates. The general performance and yield of alcoholic fermentation was not influenced by lysozyme. However, wines with delayed malolactic fermentation due to lysozyme addition contained significantly greater concentrations of free hydroxycinnamic acids but did not necessarily show elevated concentrations of volatile phenols. The reasons for this inconsistent behavior are yet unknown, but it is likely to be a side effect of lysozyme in combination with a detoxification mechanism that only occurred under specific circumstances. Additional experiments in model systems indicate that lysozyme can affect the yeast at higher pH by attacking chitin, which is incorporated in the bud scars of yeast cell walls and therefore weakens the structure of the cell. Thus, an attempt to detoxify the medium to secure cell survival could consequently cause a higher production of volatile phenols. The increased release of monomeric hydroxycinnamates in the presence of lysozyme only occurred after alcoholic and malolactic fermentation, indicating only passive involvement of the present organisms. While the influence of different concentrations of lysozyme on the concentration of free hydroxycinnamic acids could be shown in wine model systems, the exact mechanisms leading to higher concentrations of volatile phenols in only some wines remain to be clarified.

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**Rapid and Matrix-Independent Method to Analyze Anthocyanins in Red Grape Juice and Wine by FT-MIR**

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Qualitative and quantitative analysis of monomeric anthocyanins can be used to evaluate enological treatments or identify the grape variety or age of a wine. Analysis of food and beverages by Fourier Transform Mid-Infrared Spectroscopy (FT-MIR) has increased in recent years. This indirect fingerprinting method allows easy and rapid determination of multiple attributes at a very low cost per sample. In practical industry applications, the main disadvantage of FT-MIR is the necessary differentiation between juice and wine matrix because high sugar or ethanol levels have distinct effects on the accurate detection of most analytes. This often leads to defective data, especially in young and still-fermenting wines. Anthocyanins, in contrast, have a variety of properties that allow analysis independent of the matrix because they are often bound to sugars and acids, which makes the whole molecule more accessible to MIR spectroscopy. Over 300 grape and wine samples were selected, processed, and analyzed for their anthocyanin composition. Reference analysis was performed by HPLC-DAD, and the basic calibration for the FOSS FT 120 WineScan was set up with the FOSS CalibrationMaster using Partial Least Square Regression modeling. The validation and quality control tools show that several parameters like total anthocyanins, malvidin-3-glucoside, and the sum of acylated anthocyanins can be determined with sufficient accuracy as they are present in high enough concentrations. The coefficient of determination ( $R^2$ ) in relation to the reference method ranges from 0.73 to 0.85 for these param-

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eters. Other minor components, for example single anthocyanins, mostly lack that precision, although current literature suggests otherwise. However, the quality of the correlation can be improved by including more samples into the calibration. The extremely low cost of this method compensates for the possible lack of precision for industry applications.

*Funding Support: none*

### Evaluating Grape Root Architecture in a 101-14Mgt x 110R Genetic Mapping Population

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Intrinsic properties of grapevines like root system architecture are better selection criteria for developing drought-resistant rootstocks than specific physiological responses to water stress. Components of root system architecture, including rooting angle, the degree and density of branching, coarse-to-fine root ratio, and root thickness, affect the distribution of roots in the soil profile and influence root growth, seasonal development, resource acquisition and allocation, and responses to environmental conditions. Drought-sensitive commercial rootstocks possess highly branched, fibrous root systems, while drought-resistant rootstocks produce thicker roots with few lateral branches. We developed an F<sub>1</sub> population derived from a cross between the grape rootstocks 101-14Mgt x 110R that segregates for root system architecture based on a screen of five-week-old plants grown from herbaceous cuttings in sand in the greenhouse. To normalize the variation inherent to root measurements, root systems were characterized using two primary parameters: specific root length (root length  $\div$  biomass<sup>-1</sup>) and skewness, which assigns a value to the root diameter distribution in whole-root systems. Positive skewness indicates that the majority of roots within a root system possess diameters thinner than the average diameter, while negative skewness indicates abundant thick roots. We used a subset of F<sub>1</sub> progeny to validate the results of root architecture evaluations performed using the greenhouse screen with one-year-old dormant woody cuttings grown in the field in a Reiff fine sandy loam. Rapid greenhouse screens to evaluate root system architecture can aid in determining the genetic constitution of root traits and in selecting desirable architectural types for breeding complex traits like drought resistance.

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**Enology and Viticulture – CONTINUED**

**Method Optimization for Total Arsenic Analysis in Wines using HG-MP-AES**

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Arsenic (As) is found in the environment and its concentrations are increasing from natural sources and anthropogenic activities like mining, smelting, and past farming with arsenic-containing pesticides. This can cause the element to accumulate in food and beverage products. Arsenic has negative health effects and poses exposure risks to humans when contaminated food products are ingested, because the predominant arsenic exposure pathway for humans is through dietary intake. Research on food and beverage products is therefore essential to understand arsenic's role in human health. Few studies have addressed total arsenic content in wine. This poster describes the optimization of a method to determine the total As concentration in wines using an Agilent 4200 microwave plasma-atomic emission spectrometer (MP-AES). Arsenic concentration was determined via hydride generation (HG) using the Multimode Sample Introduction System (MSIS), which provides lower detection limits than conventional liquid introduction. Wine samples were collected with varying ethanol concentrations and wine styles such as red, white, rosé, sparkling, and fortified wines. The developed method provides a cost-effective approach for arsenic and elemental profiling that can be used for all wine types and alcohol concentrations.

*Funding Support: University of California, Davis, Agilent Technologies*

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**Effects of Prebloom and Fruit-set Leaf Removal on Yield, Composition, and Wine Quality of Shiraz**

**Kevin Usher**,\* Mike Watson, Pat Bowen, David Gregory, Carl Bogdanoff,  
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High-quality Shiraz wines are produced in the Okanagan Valley of British Columbia, but the crop requires extensive bunch thinning to facilitate maturation. A prebloom and fruit-set leaf removal trial was established to evaluate the effect on crop load, fruit quality, maturation, and wine quality. The four treatments were no defoliation, prebloom removal of four basal leaves, prebloom removal of six basal leaves, and fruit-set defoliation in the fruiting zone. The experiment was a randomized complete block design with five blocks. The training system was bilateral, vertical shoot-position, and vines were two-bud spur-pruned. Wines were made using conventional methods with field treatments and replicates maintained throughout winemaking, resulting in 20 wines. Basic composition and phenolics were measured in both fruit and wine. Prebloom leaf removal lowered pruning weights and the number of berries/cluster without impacting berry size or the following year's bud fruitfulness. Phenolic concentrations increased substantially, and fruit maturation was marginally advanced with six-leaf prebloom removal.

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Sensory evaluation of the wine was done blind by 16 judges tasting 10 wines twice in a balanced incomplete block design. The panel evaluated intensity of black pepper, red fruit, black fruit, jam/cooked/dried fruit, floral, meat, and vegetative flavor and aromas and astringency, body, and length of aftertaste. Sensory evaluations showed high black pepper aroma with leaf removal at fruit set and elevated black fruit aroma with all leaf removal treatments, while vegetative aroma and flavor was more abundant with no leaf removal. Wines from prebloom leaf removal were more astringent and wines from fruit-set leaf removal were least astringent. The sensory assessment of astringency was supported by a two-fold higher concentration of condensed tannins in the six-leaf prebloom treatment.

*Funding Support: British Columbia Wine Grape Council and the Agriculture and Agri-Food Canada AgriInnovation Program*

### Effect of Initiating Alcoholic and/or Malolactic Fermentations on Microbes in Wine and Its Sensory Profile

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Microbial populations and community dynamics during alcoholic and malolactic fermentations can affect the sensory attributes of the final wine. Factors influencing the microbial population include SO<sub>2</sub> addition, length of cold soak or cold settling, temperature, and yeast inoculation history. It is common practice to inoculate grape must or juice with *Saccharomyces cerevisiae* for alcoholic fermentation (AF) and *Oenococcus oeni* for malolactic fermentation (MLF). Changes in microbial populations and communities were monitored throughout a Chardonnay fermentation. At the strain level, we used a culture dependent approach, whereby strain typing was accomplished by microsatellites for *S. cerevisiae* and by VNTRs for *O. oeni*. At the species level, we used a culture-independent method using Illumina Miseq NGS in conjunction with propidium monoazide (PMA). Yeast and bacterial diversity and composition were compared among the following inoculation treatments: co-inoculation of *O. oeni* and *S. cerevisiae* in grape must, inoculation of either *S. cerevisiae* or *O. oeni* in grape must, post-AF inoculation of *O. oeni* in inoculated or spontaneous fermentations, and uninoculated wine. Bacterial populations, *S. cerevisiae* populations, yeast community diversity and composition, and chemical and sensory profiles of the finished wine were measured. *O. oeni* inoculation prior to AF resulted in lower bacterial species diversity than post-AF *O. oeni* inoculation and uninoculated wine. Spontaneous AF resulted in greater diversity of yeast, bacterial species, and *S. cerevisiae* strains. Principal component analysis of sensory data showed trends among treatments. Sensory profiles indicated that both *O. oeni* and *S. cerevisiae* inoculation changes sensory attributes of the final wine. The practice of inoculating must with yeast and bacteria impacts microbial composition, resulting in less diversity of microbes, which in turn reduces positive sensory attributes of the wine such as body and aftertaste, tropical fruit, vanilla, and oak flavors, and buttery and vanilla aromas.

*Funding Support: BC Wine and Grape Council, and Natural Sciences and Research Engineering Council (NSERC) Engage*

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Enology and Viticulture – CONTINUED

**Flowering Time and Seed Number Contribute Asymmetrically to Uneven Ripening Initiation among Fruits in *Vitis vinifera***

**Amanda Vondras**,\* Satyanarayana Gouthu, Joseph Schmidt, Anna-Rose Petersen, and Laurent Deluc

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Ripening in *Vitis vinifera* is notably asynchronous among berries within clusters. Why this occurs is not entirely understood, though differences in seed number and unequal developmental durations that arise from asynchronous flowering within a cluster have been proposed. This study examined the extent to which both factors contribute to individual fruit ripening progress by midveraison, when half of berries in a cluster have initiated ripening, and whether either or both factors affect the timing of characteristic, ripening initiation-associated changes in abscisic acid and auxin before, at, or after veraison. A decline in auxin and increase in abscisic acid are key components of ripening initiation in fruits; therefore, differences in the timing of this “switch” should correspond to differences in ripening initiation among individual fruits. Ultimately, developmental duration and flowering time did not sufficiently explain how far berries had progressed into ripening because fruits did not require a fixed amount of time to initiate ripening. Fruits from early and late flowers with similar seed number initiated ripening at the same time in spite of differences in chronological age. This suggests either that an early developmental enhancement occurred for late-initiated fruits or that flowering time is an inappropriate “day zero” and is simply a consequence of some underlying source of variation, such as asynchronous cell division or uneven cell number in floral primordia. Only seed number was linked to the timing and magnitude of ripening-related hormone changes, supporting that seeds have a comparatively larger influence than flowering time on ripening initiation in individual berries. More specifically, if the fraction of berry weight occupied by seeds was high, then the initiation of ripening for that berry, and its associated hormone changes were delayed relative to berries with less seed weight versus total berry weight.

*Funding Support: Oregon Wine Research Institute, Oregon Wine Board, Oregon State University's College of Agriculture*

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**Grape Leafroll Virus: A Systems Approach to Understand Its Interaction with the Plant and Its Effect on Fruit Ripening**

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Regulation of gene expression by transcription factors and posttranscriptionally by small RNAs and alternative splicing is an essential component of plant development and stress responses. The present study used RNA and small RNA sequencing to assemble a holistic view of these regulatory agents during ripening in *Vitis vinifera* and in response to GLRaV-3 infection. mRNA and small RNAs from healthy and GLRaV-3-infected Pinot noir fruits were measured from the onset of ripening to fruit maturity. There were ~1,700 differentially expressed genes when

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comparing healthy and infected fruits over time, with the largest number occurring at ripening onset and declining toward harvest. We identified how the virus impacts gene expression patterns by performing triclustering analyses and whether genes that experienced behavioral changes have functions and regulatory elements in common by assessing enriched gene ontology classes and *cis*-regulatory elements. In addition, some genes that were not differentially expressed were alternatively spliced due to the virus, showing that the virus not only affects gene expression but the composition of transcripts. Upon analyzing the small RNA sequencing data, we observed that 1 to 3% of small RNA reads were virus-derived, which is consistent with a targeted plant antiviral defense response. Ripening, stress, and chromatin remodeling-associated genes were predicted as targets of the miRNAs found in our data. Of the ~180 miRNAs identified, ~50 were previously annotated in grape. Finally, we used genes differentially expressed between healthy and infected plants to construct a correlation-based network to understand how the virus disrupts normal ripening and to identify core nodes through which the presence of the virus potentially induces widespread changes in the berry transcriptome via transcription factors, alternative splicing, and small RNAs. Collectively, our results suggest a complex array of regulatory and metabolic pathways through which GLRaV-3 may impact ripening.

*Funding Support: USDA, Oregon Wine Research Institute, Oregon Wine Board, Oregon State University's College of Agriculture*

### Influence of Yeast Strain on the Production of Volatile Compounds in Cabernet Sauvignon Wine

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The selection of yeast strain is critical in wine production, since yeast can preserve and even enhance grape varietal aromas. For a new wine region like Chihuahua, the selection of the optimal yeast strain for each grape variety is crucial for the growth of the wine industry. In Chihuahua, grapes grow in a desert climate with extreme day/night temperature variations during ripening; they achieve full ripeness with high concentrations of sugar and total polyphenols, making them promising to produce high quality wine. Unfortunately, there is no information about the optimal conditions for fermentation, the best yeast strain for each variety, or how yeast strain influences the overall wine aroma. Chihuahuan Cabernet Sauvignon grapes were fermented using four different yeast strains commercially available from Enartis Viquiry and Scott Laboratories. Organic volatile compounds were analyzed by headspace solid phase microextraction gas chromatography-mass spectrometry using Carbowax/Divinylbenzene and Divinylbenzene/Carboxen/Polydimethylsiloxane fibers using a DB-Wax column. Each strain produced individual volatile compounds in different concentrations and combinations, the most abundant being esters and alcohols with fruity and floral aromas. The results of this work are important for the Mexican wine industry. Information on the concentrations of volatile compounds depending on yeast strain will help increase

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wine aromatic quality and create a foundation for future research on other grape varieties grown in Mexico.

*Funding Support: Secretaría de Agricultura, Ganadería, Desarrollo rural, Pesca y Alimentación*

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**Comparison of Different Screening Techniques for Grape Powdery Mildew Resistance**

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Virtually all *Vitis vinifera* cultivars are highly susceptible to grape powdery mildew, which is caused by the biotrophic fungal pathogen *Erysiphe necator*. Discovery of new and unique resistance loci and their use in breeding programs will lead to sustainable and economic control of powdery mildew. Breeding populations can be used to generate genetic maps and to associate traits such as resistance with genetic markers, which can then be used as rapid and powerful tools for selection. While genetic mapping and DNA analysis can be semi-automated and generalized among crops, measuring or describing the trait of interest (phenotyping) is crop- or disease-specific. Developing reliable and rapid phenotyping systems is critically important for breeding and for developing accurate genetic markers. We compared three screening assays for grape powdery mildew resistance: field evaluations, a greenhouse assay with natural and supplemental powdery mildew inoculum, and a detached leaf *in vitro* assay. All three systems were measured using the one to six categorical scale devised by the International Organization of Vine and Wine (OIV). Data from the *in vitro* leaf assay was also compared with qRT-PCR by measuring the relative cDNA of the fungal tissue. All methods were assessed on a F<sub>1</sub> breeding population from a cross between susceptible *V. vinifera* F2-35 and resistant *V. piasezkii* DVIT2027. The variance and correlation among phenotype scores and the impact of different methods on detecting a resistance locus on a genetic map were evaluated. We concluded that scoring powdery mildew symptoms on detached leaves under a microscope is the most rapid and effective way to evaluate a segregating population.

*Funding Support: Comparison of Different Screening Techniques for Grape Powdery Mildew Resistance*

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**Identification of Vineyard and Winery Bacteria and Their Impact on Problematic Fermentations**

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The role of grape and winery bacteria in inhibition of yeast fermentation has not been well defined. Industry samples of chronically difficult-to-ferment juices and arrested wines from the 2013 to 2015 vintages were sought from winemakers. Over 200 wines were obtained from 55 wineries, many of which displayed high bacterial levels. In 2013, one winery submitted four samples of hard-to-ferment

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juice and their resulting wines. All juices and 25% of the wines had bacterial contamination, including *Gluconobacter*, *Acetobacter*, and *Lactobacillus* species. The 2014 vintage yielded increased rates of problematic fermentations and 127 wines were submitted from 40 wineries. Twelve percent of these wines had culturable problematic bacteria, including three species of acetic acid bacteria, two *Lactobacillus* species, and one *Pediococcus* isolate. Eighty-seven wines from 18 wineries were received from the 2015 vintage, of which six to date contain acetic acid and lactic acid bacteria. In 2015, vineyard and must samples were taken on-site from three wineries that had submitted samples in previous years to determine whether the bacteria were coming from their source vineyards or were residents of the winery. Bacteria were cultured and isolated from samples and identified using 16S ribosomal DNA sequencing. Additionally, bacterial isolates were tested for their potential to induce the [GAR<sup>+</sup>] prion, a metabolic adaptation in yeast that can contribute to slow or stuck fermentation. Of the seven vineyards sampled, three contained at least four distinct lactic acid bacterial species. Preliminary results support the hypotheses that bacteria persist in wineries from vintage to vintage and that some organisms may be harbored on grapes in the vineyard. Bacteria previously isolated and identified from stuck fermentations have also already been shown to either inhibit yeast growth or promote the prion-induced [GAR<sup>+</sup>] state that reduces yeast affinity for glucose.

*Funding Support: American Vineyard Foundation*

### Effect of Various Levels of Biochar and Compost on Growth of Valvin Muscat Grapevines in a Controlled Environment

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Increasing demand for productive vineyard soils is driving growers to attempt new agricultural practices. Many such methods use renewable, regionally available resources. Biochar is a highly porous soil amendment created by burning organic matter under low-oxygen conditions. By using renewable soil amendments such as biochar, the health and yield of vines can be improved. The aim of the research was to determine the efficacy of using biochar to promote grapevine development. One-year-old bare-root cuttings of Valvin Muscat (NY62.0122.01) grafted onto 1103P were grown in a glasshouse in substrates containing either: (1) 30 B: 30% biochar + 70% medium, (2) 70B: 70% biochar + 30% medium, (3) 100B: 100% biochar, (4) 30C: 30% compost + 70% medium, (5) 70C: 70% compost + 30% medium, (6) 100C: 100% compost, (7) 15B15C: 15% biochar + 15% compost + 70% medium, (8) 35B35C: 35% biochar + 35% compost + 70% medium, (9) 50B50C: 50% biochar + 50% compost, or (10) 100% medium. The pH and EC of the substrates, optical estimate of leaf chlorophyll content, leaf gas exchange, true leaf number, lateral leaf number, shoots lengths, and lateral and true leaf area were measured and root and shoot dry weight was determined. Biochar induced high pH (>8) if present in the mixes at over 35%. Biochar and compost combined at from 15 to 35% biochar and 15 to 35% compost induced more chlorophyll than the control. Photosynthetic carbon assimilation, stomatal conductance, and intercellular CO<sub>2</sub> concentration did not differ among treatments; however, positive correlations were found between photosynthetic carbon assimilation and

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optical measurement of chlorophyll concentration. Mixes with 30B, 15B15C, and 35B35C increased leaf area, but total biomass was similar to the control. Preliminary findings indicate that biochar in combination with compost can be used to promote grapevine development.

*Funding Support: Missouri Wine and Grape Board*

**Comparison of Solid Phase Extraction and Liquid-Liquid Extraction for Aroma Extract Dilution Analysis of Merlot Wine**

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Wine aroma is one of the most important attributes for wine quality. To understand the volatile compounds responsible for wine aroma, wine is typically extracted with an organic solvent using liquid-liquid extraction (LLE), then analyzed by gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA) to determine the most important compounds. This protocol requires large quantities of organic solvent. The extracts also contain high levels of alcohols and acids that interfere with analysis. A simple Lichrolut-EN resin-based solid phase extraction was compared to traditional LLE to study the aroma compounds in Merlot wine. The extracts were passed through a solvent-assisted flavor evaporation device and analyzed by AEDA. In general, SPE was comparable to LLE in obtaining aroma extracts suitable for GC-O analysis, but it was faster and used much less organic solvent. SPE extract had higher flavor dilution values than LLE for esters and lactones but lower values for acids, alcohols, phenolics, and sulfides. AEDA detected ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl isovalerate, ethyl octanoate, isoamyl acetate, 2,3-methyl-1-butanol,  $\beta$ -damascenone, butanoic acid, 3-methylbutyric acid, hexanoic acid, vanillin, guaiacol, eugenol, 4-vinylphenol, 4-vinylguaiacol, 4-methylguaiacol, *cis/trans*-whisky lactone,  $\gamma$ -nonalactone, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, homofuraneol, sotolon, methional, 3-(methylthio)propanol, 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, and 3-hydroxy-2-butanone as the most important odorants for Merlot wine.

*Funding Support: Oregon Wine Research Institute, China Scholarship Council*

**Investigating the Effect of Topography on Vine Water Use and Vine Stress**

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While evapotranspiration (ET) and crop coefficient ( $K_{cc}$ ) information for winegrapes grown on flat terrain are widely published, there is little information for vines grown on slopes. We measured ET and stem water potential (SWP) in sloped vineyards to evaluate differences in vine water use; our goal was to improve irrigation management on the basis of topography. Site A (10 May to 12 Oct 2013) was Merlot on Chenin blanc root, planted in 1973 in a granitic soil on a 10% slope. Site B (8 Aug to 10 Nov 2014 and 8 April to 18 Oct 2015) was Cabernet Sauvignon on 3309 rootstock, planted in 2000 on metasedimentary soil with an 18% slope. On each slope, the ET was determined using the residual of

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the energy balance method and eddy covariance to measure sensible heat flux.  $ET_c$  from local CIMIS stations was used with the measured  $ET$  to calculate crop coefficients. Midday SWP measurements were taken periodically. Vine SWP became more negative as  $K_c$  and  $ET$  decreased. SWP was similar for vines in the north and south blocks at site A, but became more negative in the north-facing block at site B in both years. At both sites in 2014, greater daily net radiation and  $ET$  was observed on the south-facing slope; at site B in 2015, greater daily net radiation and  $ET$  was observed on the south-facing slope in spring and fall and on the north-facing slope in June. At both sites, the cumulative vine water use ( $ET$ ) was greater on the south slope than the north, and cumulative  $ET$  on both slopes was greater than measured precipitation + applied irrigation. We conclude that vines on south-facing slopes used more water due to higher net radiation; however, factors contributing to vine stress are complex and require specific site evaluation.

*Funding Support: In-kind from authors*

#### Effect of Regulated Deficit Irrigation on Malbec and Syrah Grape Volatiles

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The concentrations of volatile compounds and precursors in grape berries are highly influenced by viticultural practices. Among these cultural practices, regulated deficit irrigation to improve water use efficiency and reduce canopy vigor is an important practice for sustainable agriculture, especially in arid and semi-arid areas. Imposing a water deficit to the vine during berry development is also an important vineyard management strategy to alter grape and wine quality. Previous studies showed that water deficit influenced physiological parameters of the vine, changed berry composition, and improved sensory attributes of wines by increasing fruity aromas and decreasing vegetal aromas. The objective of this study was to determine the effect of water stress on grape secondary metabolites and its implications for wine quality. Four irrigation regimes (70%  $ET_c$  from fruit set to veraison, 35%  $ET_c$  from veraison to harvest [70/35]; 70%  $ET_c$  sustained from fruit set to harvest [70/70]; 35%  $ET_c$  from fruit set to veraison, 70%  $ET_c$  from veraison to harvest [35/70]; and 35%  $ET_c$  sustained from fruit set to harvest [35/35]) were applied to the vines with two irrigation frequencies (1x = one event per week and 3x = the same irrigation amount apportioned into three irrigation events per week). In 2013, the 35/35 treatment with 1x frequency resulted in the greatest *trans*- $\beta$ -damascenone concentration in Malbec grapes, but no significant difference was observed in Syrah grapes. The 35/35 treatment at 1x frequency also resulted in more vitispirane and TDN in Malbec grapes but not in Syrah. Syrah had higher concentration of  $C_6$  compounds than Malbec. The effect of irrigation on grape volatile composition may depend on variety. In 2014, wine was made from the trial grapes and wine volatile composition was analyzed. The concentration of *trans*-3-hexen-1-ol was highest with the 70/35 and 70/70 treatments in both Malbec and Syrah wines compared to the 35/70 and 35/35 treatments.

*Funding Support: USDA-CSREE-NWSFR*

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## Industry

### Alternative to Filtration or Chemical Stabilization for Microorganism Removal: Chitosan Fining

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Microbial contamination and its consequences are major threats to wine quality. Capable of growing under difficult conditions at any time during winemaking, spoilage microbes are opportunist organisms that are very difficult to control and eliminate. They are responsible for chemical and physical changes in wine which lead to loss of quality. The development of spoilage microorganisms is usually noticed by the presence of off-characters when their population is at the exponential phase and the wine is already damaged. Early detection of spoilage microorganisms allows winemakers to be proactive and keep them under control. Maintaining good cellar hygiene, minimizing spoilage microorganisms in juice, controlling alcoholic and malolactic fermentation, and protecting wine during aging are essential to manage microbe populations. Recent developments offer winemakers new tools to remove undesirable microorganisms through fining, thereby avoiding filtration and reducing the use of antimicrobial chemicals. Chitosan is a natural and non-allergenic polysaccharide that is produced by de-acetylation of chitin, a polysaccharide produced from *Aspergillus niger*. It interacts with microorganisms via charge attractions and degrades their cell walls, leading to cell death. Fining with chitosan removes the most notorious and feared spoilage microbes from juice and wine: *Brettanomyces*, *Zygosaccharomyces*, *Lactobacillus*, *Pediococcus*, *Oenococcus* and even *Acetobacter*. Chitosan can be used as a preventive or curative tool to eliminate spoilage microorganisms. Trial results showed that depending on the winemaking stage, different types of chitosan formulations should be used.

*Funding Support: Enartis*

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### Innovative Tools to Limit Juice Oxidation

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Juice oxidation, which causes loss of aromatic precursors and primary aromas, browning, and off-flavor development, is of great concern to winemakers. Limiting oxygen contact, working at cold temperatures, and using antioxidants such as sulfur dioxide or tannins can inhibit oxidative enzymatic reactions. Metals such as copper, iron, and aluminum are co-enzymes and catalyzers of oxidation reactions in juice and wine. They usually come from pesticides, winemaking equipment, copper sulfate treatment, or even accidental must contamination. This study focuses on removing metals from juice to reduce oxidation reactions and preserve wine quality. PVI-PVP, a copolymer of vinylimidazole and vinylpyrrolidone, binds to heavy metals selectively with its functional imidazole groups.

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Several studies have proved the efficacy of PVI-PVP on removing metals in must and wine. This presentation focuses on the synergistic effect of PVI-PVP combined with selected yeast derivatives to remove metals and inhibit juice oxidation. The results of 2015 trials in wineries around the world showed that application of this blend at the beginning of fermentation protects wine from browning and improves aroma complexity and stability.

*Funding Support: Enartis*

### Application of Automated 4-D High Resolution Vineyard Soil Hydrology Assessments and Visualization

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A visual information environment for effective agricultural management and sustainability (VINES) was applied to commercial winegrape vineyards in California to sustain their appellation-specific grape and wine qualities and yields under both irrigated and dry-farmed conditions. The system was designed to validate, refine, and improve the automatic landform inference mapping (ALIM) soil modeling/sampling method and to define key components for general perennial crop production and winegrapes in particular. The validity of this technology has been tested through analysis of data collected through sensor deployment in North Coast vineyards and through development of highly-resolved, 4-D vineyard maps that visualize soil composition, vine water availability, vine nutrient status, and subsequently, grape maturity and juice composition. A comparison of predicted map-based water flow at several depths and locations against in-field, sensor-sampled values was conducted. The accuracy of predicted soil characteristics across vineyard blocks at several locations was validated based on physical and chemical analyses and statistical comparisons. The first completed, real-time spatial soil functional maps were used to design visual analytics to create an effective decision-making environment applicable in commercial vineyards. Working directly with vineyard managers and winemakers, this integrated research and extension project collaboratively developed an interactive, user-driven decision-making environment that employs visual analytics to organize all inputs from soil sensors, high-resolution spatial soil function, and water dynamic responses, while integrating available historic and current data flows. VINES is designed to integrate future soil, plant, viticulture, and enological models into its decision support system to help respond to changing climatic conditions, particularly drought, and to improve general vineyard management, harvest scheduling, and long-term sustainability and life cycle decisions.

*Funding Support: National Science Foundation*

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**Strategies to Limit Non-Enzymatic Oxidation and Extend White Wine's Shelf Life**

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The forces at work in wine oxidation and their effects on wine quality have long fascinated researchers. In non-enzymatic oxidation, oxygen does not react directly with phenolic compounds, but through a reaction catalyzed by transition metals (Cu<sup>+</sup> and Fe<sup>+</sup>) that convert oxygen into a highly reactive radical, capable of oxidizing organic compounds. Oxidation of phenolic compounds in white wines causes premature aging, browning, and pinking, leading to wine deterioration and loss of quality. Adopting strategies that block these systems is an effective way to improve wine aging potential and shelf life. These strategies include removing metals (catalyzers of oxidation), reducing the concentrations of phenolic compounds in wines (precursors to oxidation), and controlling the redox potential (decreasing the risk of oxidation). Enological products containing PVI-PVP were evaluated during winemaking for their effect on wine oxidative potential. PVI-PVP is an adsorbent polymer (copolymers of vinylimidazole and vinylpyrrolidone) that is capable of removing from wine heavy metals such as copper (Cu), iron (Fe), and aluminum (Al). PVI-PVP can also bind to phenolic compounds that could otherwise enter into oxidative reactions. This double bonding ability makes this polymer useful in blocking the oxidative system. This study showed that PVI-PVP was effective at removing metals, although more copper was removed than iron. The efficiency of metal elimination directly correlated with dosage rate and contact time. Additional testing using an accelerated-aging protocol showed that wines treated with PVI-PVP had a lower oxidation potential and developed less brown hues.

*Funding Support: ESSECO group*

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**Aroma Enhancement in White Wines during Fermentation and Storage Due to New  $\beta$ -Glycosidase Enzyme Activities**

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A major portion of primary wine aroma substances are terpenes. In grape berries, they are primarily located in the skin as sugar-bound monoterpene precursors (glycosides). In this glycosidically bound form, they are neutral in smell: only the respective aglycone is odor-bearing. By enzymatic cleavage of the sugars (monosaccharides or disaccharides), the corresponding monoterpene alcohol (such as linalool or geraniol) is liberated and becomes organoleptically perceptible. Monoterpene alcohol and sugar residues are linked by a glycosidic bond; thus, enzymatic breakdown is carried out by  $\beta$ -glycosidases. These enzymes are differentiated according to their specific activities, which depend on the type of sugar residue to which the terpenes are linked (e.g., arabinose, rhamnose, or apiose). In

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the course of this project, enzymes with new  $\beta$ -glycosidase activities were evaluated. These enzymes were not inhibited by high glucose concentration and promote a significant increase in aroma release.

*Funding Support: Erbsloeh Geisenheim AG, Erbsloehstrasse 1, Geisenheim D-65366, Germany; Institute of Viticulture and Enology Freiburg (WBI), Merzhauserstrasse 119, 79100 Freiburg, Germany*

### Evaluation of Winegrape Ripeness using a New Combined Brix and Acidity Meter

**John Thull**, Geoffrey Anderson\* and Matthew Clark

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Proper fruit ripeness is a primary quality predictor in winemaking. A simple, portable meter to monitor both fruit sugar and acid in the vineyard could provide this timely, important information. The PAL-BX/ACID 2 combo meter from ATAGO-USA is evaluated for utility as a tool to monitor preharvest ripeness of winegrapes. Grape sugar is measured by digital refractometry directly from juice and total acid, by conductivity following 50-fold dilution. The evaluation, performed within the University of Minnesota Grape Breeding program, included several varieties of *Vitis vinifera* and hybrid grape. Brix and acid changes were tracked over a two-month period prior to harvest. The meter was easy to use and produced repeatable results. Acidity is expressed as tartaric acid equivalents (g/L) and when combined with sugar (Brix) measurements, the meter offers a rapid, in-field analysis of fruit ripeness that is useful to optimize harvest timing. The interpretation of acid conductivity versus titratable acidity data is discussed.

*Funding Support: University of Minnesota Grape Breeding Program*

### Development and Validation of a Reagent Based on Enzymatic Reactions for Determining Histamine in Wines

Sabina Duenas and **Andreu Tobena\***

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The presence of histamine in wine is becoming increasingly important to consumers and producers alike, due to the health implications of its potential toxicity to humans. Histamine also has the potential to be an indicator of unsanitary conditions during wine production. Current methods for analyzing histamine are HPLC, ELISA, and fluorimetry. These methods require expensive and sophisticated instrumentation and, as a consequence, skilled technicians. We present a new, simple, and rapid enzymatic method for determination of histamine in red and white wine based on the specific reaction of histamine with recombinant histamine dehydrogenase from *E. coli*, reducing a soluble tetrazolium salt to form a

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formazan salt that absorbs at 420 nm. This can be measured using visible spectrophotometry and correlated, through calibration, with histamine concentration. A simple method to remove interfering substances (reducing agents like polyphenols and anthocyanins) from wine samples is also presented. This new reagent is stable in a ready-to-use liquid form, avoiding end-user influence. It has been formulated for use in any photometer or automated analyzer. The assay's linearity, limit of detection (LoD), limit of quantification (LoQ), repeatability, within-laboratory reproducibility, trueness, and recovery were characterized using a BioSystems Y15 automated analyzer and HPLC method OIV-MA-AS315-18 as the reference.

*Funding Support: BioSystems S.A.*

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**Analysis of Toast Profiles by Near Infrared Spectroscopy for Traditional Barrels and Alternative Oak Products**

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Inconsistency in fire-toasted barrels has been problematic for winemakers throughout winemaking history. Variance in toast profiles from a single cooper can cause unanticipated variance in resulting flavor profiles. To address the cooper's dilemma, near infrared (NIR) spectroscopy was investigated for use as a tool after toasting to measure the magnitude of heat treatment for common fire-toast profiles. NIR spectroscopy is frequently used in the wood and paper industries as a viable, rapid, and inexpensive means to quantify wood constituents and aroma and flavor precursors: cellulose, hemicellulose, lignin, and extractives. Spectra were collected on different convection-baked oak profiles: 225-L American oak barrels with 27-mm staves and 225-L French oak barrels with 22-mm or 27-mm staves. Principal component analysis (PCA) of the NIR spectra revealed distinguishing spectral features for convection-baked treatments and fire-toasts of blonde, medium, medium plus, and heavy. The first two principal components explained over 95% of the variance across toast profiles. Using PCA, barrel-to-barrel variation was quantifiable and was greatest for moderate-toast levels. Differences in raw wood composition and variance in production were likely contributors to spectral differences within toast levels. Analysis of toasting magnitude for traditionally toasted barrels by NIR was deemed feasible and reliable as a quality assurance method to assure winemakers of toasting uniformity.

*Funding Support: Cork Supply USA*

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