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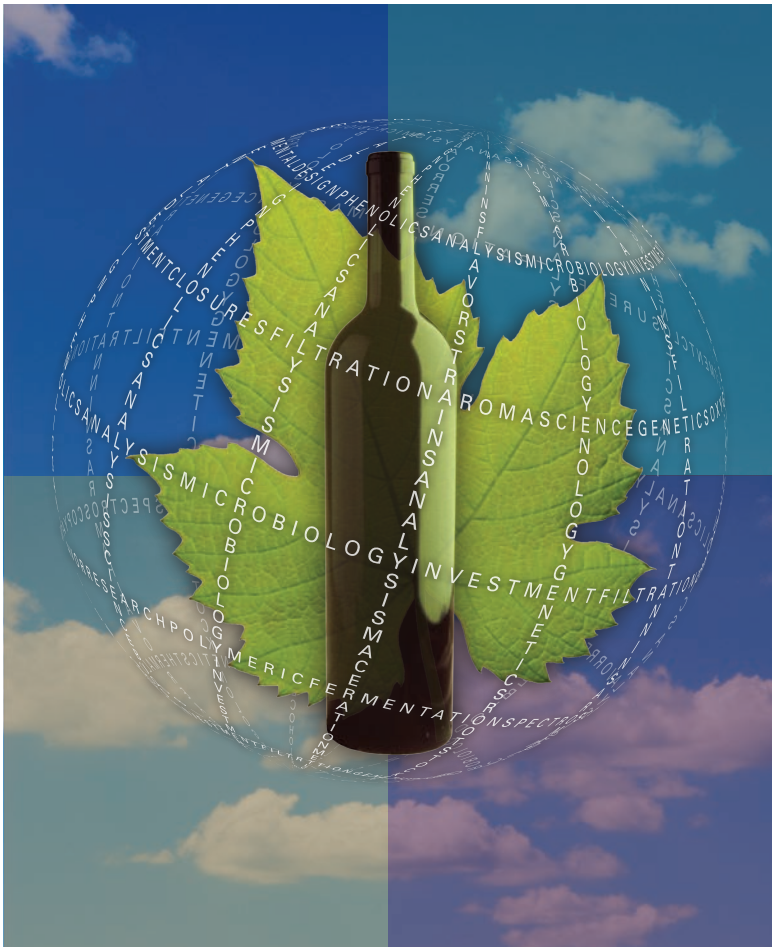


## TECHNICAL ABSTRACTS

**69th ASEV National Conference**

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**Portola Hotel & Monterey Conference Center**

Monterey, California USA

AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

# 2018 National Conference Technical Abstracts



## Oral Presentation Abstracts

### Wednesday, June 20



### Thursday, June 21



## Poster Presentation Abstracts

### Wednesday, June 20 & Thursday, June 21

## Alphabetical Listing of All Authors with Submitted Abstracts



*Abstracts are listed in the approximate presentation order as scheduled in the conference program.*

## Enology — Wine Phenolics Session

### Influence of Berry Ripeness on the Extraction of Skin and Seed Polyphenols and Sensory Characteristics of Pinot noir

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Much of the art of red winemaking is in controlling the extraction and development of grape phenolics. The objective of this study was to investigate the influence of berry ripeness on extraction of seed and skin polyphenols during fermentation/maceration of Pinot noir. Grapes with high and low ripeness were pressed gently, and the pomaces of both ripeness stages were sorted into two fractions: skins and seeds. The obtained fractions were reconstituted in the juice in original and changed proportions. Analytical discrimination of seed and skin polyphenols was by LC-MS fingerprinting. During 14 days fermentation/maceration on low-ripe skins and low-ripe seeds, a disproportionate increase in seed polyphenols was observed. The wines were characterized by high content of gallic acid, procyanidin, procyanidin gallate, catechin, and epicatechin. Descriptive analysis of this wine revealed high bitterness, green tannin perception, and low color intensity. Interestingly, the addition of high-ripe skins to the ferment could compensate for the negative impact of low-ripe seeds. Anthocyanin and prodelphinidin concentrations increased; procyanidins, catechin, and epicatechin concentrations were significantly lower when low-ripe seeds were combined with high-ripe skins. Wines from this artificial crossover were described as having high color intensity, low bitterness, and full-bodied, almost like wines made from high-ripe seeds. These observations suggest that higher berry ripeness particularly increases the extractability of skin polyphenols, and that skin polyphenols react with seed phenols. Similarly, the effect of 50% removal of low-ripe seeds was independent of skin ripeness; these wines generally showed poor color stability and green tannin impression, even in wines made from high-ripe skins. It is suggested that reactions between seed and skin polyphenols are crucial for color and mouthfeel of a Pinot noir and that seeds are essential in red winemaking, even if berries are fully ripe.

*Funding Support: Research Association of the German Food Industry (FEI)*

### Characterization of Skin Cell Wall Material from Pinot noir and Cabernet Sauvignon from Different Regions

**Cristina Medina-Plaza**, Nick Dokoozlian, Ravi Ponangi, Tom Blair, David Block, and Anita Oberholster*
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The phenolic component of red wine is responsible for important elements of the flavor, mouthfeel, and quality of the finished wine. While the origin, concentration, and chemistry of the phenolics in a finished red wine are well known, the fundamental mechanisms and kinetics of extraction of these compounds from grape skins and seeds during red wine fermentation are poorly understood. Berry skin cell walls play a very important role during the winemaking process by forming a barrier to the release of important components impacting aroma and color

and by providing a potential adsorption surface. Commercial winery observations that polyphenol extraction levels during winemaking may vary based on grapegrowing region and cell wall composition are a potentially important factor influencing this relationship. In this work, the composition of skin cell wall (CW) material from *Vitis vinifera* cv. Cabernet Sauvignon and Pinot noir has been studied, aiming to relate the difference in CW composition among varieties with the polyphenol extractability obtained from different regions within California. This study includes the analysis of total soluble sugars, proteins, non-cellulosic glucose, Klason lignin, cellulosic glucose, total polyphenolic content, and lipids, as well as the isolation efficiency. Differences in the cell wall material composition between regions were found for all the parameters studied, especially for protein, non-cellulosic glucose, soluble sugars, and polyphenolic content. Multivariate analysis was performed to associate the cell wall material composition to the extractability values. Results showed that the differences in extractability could be due to differences in grape cell wall pectin, cellulose, and protein content, which are a function of the region of grape cultivation.

*Funding Support: E&J Gallo Winery*

## Impact of Fruit Maturity and Extended Maceration on Phenolic Extraction of Cabernet Sauvignon Wines

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Extended maceration is a technique used by winemakers to increase phenolic extraction in wines. While this technique is typically used for short periods of time, recent winemaking trends have given rise to extraordinary macerations, up to eight to nine months.In this study, Cabernet Sauvignon was picked at three different maturities, representing unripe, ripe, and overripe fruit. Control and extended maceration tanks were fermented in triplicate (140 L scale) and extended maceration wines were allowed to age on the skins for up to eight months. Samples were collected throughout maceration, and at each time point, samples were aged at elevated temperatures to simulate aging. Accelerated aging was used to predict phenolic changes over time if the wine was pressed after any length of maceration. At each maturity, tannin extraction reached a maximum after 60 days maceration. After 60 days, all extended-maceration wines had a similar tannin concentration, although wine alcohol levels were significantly different. At each harvest, concentrations of color-related phenolic compounds followed similar trends over time. Anthocyanin content decreased postfermentation in both control and extended-maceration wines, but the decrease was slightly greater in extended-maceration wines. While extended maceration did not increase the anthocyanin concentration, it did alter wine color (as observed by tristimulus color measurements), due to the formation of polymeric pigments. Phenolic hydrophobicity, measured to determine phenolic structural differences, depended on maturity and time, but not winemaking treatment. During accelerated aging, large polymeric pigment increased with maceration time as tannin concentrations increased. However, small polymeric pigment followed similar trends regardless of maceration time. Overall,



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## Enology — Wine Phenolics Session – CONTINUED

extended maceration increased tannin extraction and large polymeric pigment formation across all harvest dates.

*Acknowledgments: Richard Larsen, Maria Mireles, and WSU graduate students and interns are thanked for winemaking assistance and harvest laboratory analysis.*

*Funding Support: Funding for this project was provided by the Washington Grape and Wine Research Program and the National Institute of Food and Agriculture.*

### Wine Oxidation: Analysis of Quinone Reaction Products Using $^{13}C$ Isotope Tracing

**Andrew Waterhouse**,* Lingjun Ma, Christoph Bueschl, and Rainer Schuhmacher
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Quinones are key reactive intermediates formed during wine oxidation that have a key impact on wine characters, degrading color and flavor. They can readily react with nucleophiles like $SO_2$ and glutathione. Some nucleophiles have been reported to react with quinones in model wines, but some of the corresponding products have not been confirmed in real wines, and questions remain regarding which products do, in fact, arise in wine. Here, a stable M+6 isotope labeling approach was used with liquid chromatography coupled to time-of-flight mass spectrometry. By finding products with the unique isotopic ratio (M+6), it is possible to demonstrate which substances in the wine were produced by reacting with the quinone. A list of predicted products based on known wine nucleophiles was prepared and compared to the data extracted that was filtered based on the M+6 isotopic pattern using a program called MetExtract. This yielded a list of 17 compounds. Reactions were shown to occur with glutathione, methanethiol, bisulfite, and numerous flavonoids, particularly anthocyanins, and with complex products with multiple new bonds. This labeling technique can reveal real products in a very complex system and demonstrates a means to further probe the complex oxidation reactions in wine.

*Funding Support: American Vineyard Foundation*

### Pigmented Tannin and Derivative Analysis by a Complementary Suite of Mass Spectrometric Techniques

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A combinatorial prediction of the pigmented tannin in red wine was made using known proanthocyanidin and anthocyanin precursors, then following established reaction mechanisms for pigmented tannin such as pyranoanthocyanins, pinotins, portisins, and similar compounds, creating a database of postulated compounds. Formulae were derived to limit the list to only those compounds that were

## Enology — Wine Phenolics Session – CONTINUED

distinguishable by mass spectrometry. Analysis of 1.1 million compounds below 5000 Daltons showed that all could be distinguished by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR), but less precise techniques would leave some adjacent signals indistinguishable. Pigmented tannin isolates from red wines spanning more than a decade were purified on Sephadex LH20, then analyzed by FTICR and separately by a custom-made diol (normal phase) separation with a Quadrupole Time of Flight tandem mass spectrometric analyzer (nano-HPLC-QTOF). FTICR analysis obtained several thousand signals that could be matched to the database of postulated pigmented tannin derivatives, and the unknowns were cataloged for future $MS^n$ investigation. Nano-HPLC-QTOF successfully resolved several hundred peaks from the previously indistinguishable hump that results from red wine phenolic chromatography, and fragment analysis provided isomeric discrimination. Both FTICR and QToF offer high sensitivity and selectivity, providing greater signal density for the samples analyzed. With FTICR contributing very high mass accuracy for molecular formula determination and QToF fragmentation allowing discrimination of isomers, the pairing of these two techniques reveals extraordinarily detailed insights into the composition of red wine phenolics. This complementary suite of mass spectrometric techniques provides a framework for continued evaluation of real wine samples with some of the most exhaustive qualification to date and a basis for advancing the structural analysis of unknown compounds at the trace level.

*Funding Support: American Vineyard Foundation*



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## Viticulture — Disease Session

### Identification of the Pierce's Disease Resistance Locus *PdR2* from the Mexican Grape Species Accession b42-26

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A successful breeding program relies on sources of resistance from multiple, diverse backgrounds to develop resistant varieties. This lets breeders stack major and minor resistance loci through conventional breeding to ensure long-lasting resistance in the field. In this study, we identified Pierce's disease (PD) resistance in b42-26, a hybrid form of *Vitis arizonica* and *V. girdiana* obtained by Harold Olmo in Loreto in Baja, California. The F1 breeding population of 352 seedling plants was developed by crossing the susceptible *V. vinifera* (F2-35) with b42-26. A genetic map was developed with simple sequence repeat (SSR) markers. The level of polymorphism in b42-26 was very low, perhaps due to its isolation on the Baja California peninsula, which may have resulted in an inbred genetic background. The genetic map was developed with 191 SSR markers and grouped to 18 chromosomes. Chromosome 19 was not represented due to b42-26's homozygosity, even though we tested the population with 45 SSR markers associated with that chromosome. All seedling plants were phenotyped for PD resistance in the greenhouse and data were analyzed with MAPQTL v. 6.0. The analysis located resistance on chromosome 8 and this was verified in the pBC1 and pBC2 populations. The resistance locus was named *PdR2* and it resides between markers FAM82 and VMC 7h2. We are employing closely linked markers to allow the PD winegrape breeding program to stack the *PdR1b* and *PdR2* loci together, thus broadening PD resistance.

*Funding Support: CDFA PD/GWSS Board*

### Effects of *Grapevine Leafroll-Associated Virus 3* on Cabernet franc Fruit Yield, Composition, and Wine Quality

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*Grapevine leafroll-associated virus 3* (GLRaV-3) infections are spreading within and among vineyards in the southern Okanagan Valley, British Columbia. Understanding the epidemiology and economic impacts of GLRaV-3 infection will help producers make decisions regarding vineyard management, replant scheduling, and winemaking. The effects of GLRaV-3 infection on vine performance were determined in a Cabernet franc vineyard near Osoyoos, BC, over four years beginning in 2013. Symptomatic vines were verified as GLRaV-3 infected, and flanking asymptomatic vines as non-infected, each year using reverse-transcriptase PCR test. Twenty infected vines and their two flanking non-infected vines were evaluated for growth and fruiting performance. Fruit was harvested from all vines on the same day each year. There were no effects of GLRaV-3 infection on pruning mass or yield, and effects on yield components were small and inconsistent among years. Averaged over years, infection reduced berry soluble solids (SS)

at harvest by 1.2 Brix, increased TA by 0.38 g/L, and reduced the anthocyanin concentration in berry skin by 5%. In 2014 and 2015, single-vine wines were made from 10 randomly selected sets of infected and flanking non-infected vines. In 2014, when berry soluble solids averaged 26.8 and 27.6 Brix for infected and non-infected vines, respectively, infection led to wines with lower total phenolics, less black fruit flavor, more vegetative flavor, and less body and aftertaste. In comparison, in 2015, when the harvested fruit was more mature, with SS averaging 28.2 and 29.0 Brix for infected and non-infected vines, respectively, infection had less influence on wine quality, causing only reduced red fruit aroma. These results indicate that GLRaV-3 infection effects on wine quality may depend on fruit maturity at harvest. In a year when harvested fruit was very mature, infection had little influence on wine sensory quality.

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

### Red Blotch Disease Affects Vine Metabolism before Symptoms Are Apparent

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Grapevine red blotch-associated virus (GRBaV) infection can negatively impact primary and secondary metabolism of grapevines, leading to a delay in fruit development. Cabernet Sauvignon/110R vines grown in Oakville, CA, were tested in 2017 using qPCR and primers specific to the GRBaV genome sequence. Vines were classified as GRBaV(-) or GRBaV(+). Experimental vines were free from other common virus infections. Leaf gas exchange and midday stem water potential were monitored at two-week intervals from fruit set through harvest. Berry flavonol and anthocyanin composition were monitored from veraison to harvest and characterized with $C_{18}$ reversed-phase HPLC. Approximately 30 days before onset of symptoms, midday SWP was greater in GRBaV(+) vines, which was also observed at harvest. The only reduction of net carbon assimilation due to infection was observed at harvest. Likewise, few differences in leaf stomatal conductance and sub-stomatal $CO_2$ concentration were observed. Despite these observations, TSS and TA of fruit from infected vines were consistently reduced across the season; this response was not observed for pH. At harvest, TSS and TA of GRBaV(+) vines were both reduced by 16% when compared to GRBaV(-). Delayed harvest (by 1.5 weeks) did not allow improvement in TSS, which was 7% lower in fruit from infected vines. Components of yield were not affected by the presence of GRBaV. Development of anthocyanins lagged across the season by up to 8 mg/g SDM, but by harvest no differences in total amount or forms were observed. GRBaV negatively affected secondary metabolism before symptoms were apparent and fruit development was delayed as early as veraison.

*Funding Support: American Vineyard Foundation*



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## Viticulture — Disease Session – CONTINUED

### Impact of Red Blotch Disease on Grape and Wine Composition and Quality of Three Varieties Harvested Sequentially

**Raul Cauduro Girardello**, Arran Rumbaugh, Monica L. Cooper, Larry Lerno, Rhonda J. Smith, Charles Brenneman, Anji Perry, Hildegarde Heymann, Pauline Lestringant, Anita Oberholster, Kaan Kurtural, and Cassandra Plank*
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The impact of red blotch (RB) disease on grape and wine quality has been of great concern in the United States since its identification in 2011. We studied the impact of RB disease on Cabernet Sauvignon, Chardonnay, and Merlot grape and wine composition over three years. Results from the 2014 and 2015 seasons indicated that RB disease can impact grape composition by decreasing sugar concentration and delaying color development, which resulted in lower ethanol and anthocyanin concentration in their respective wines, thus affecting sensory properties. For this reason, in the 2016 season, symptomatic grapevines were harvested sequentially at two time points: (1) at the same time as healthy vines but at lower Brix, and (2) later, when Brix was similar to that of healthy vines. The aim of this study is to evaluate the impact of RB disease on grape and wine composition and sensory properties. Cabernet Sauvignon, Chardonnay, and Merlot berries from Napa Valley, Sonoma County, and San Luis Obispo, respectively, were collected weekly from veraison until harvest. Brix, pH, titratable acidity (TA), phenolic composition by protein precipitation assay, and RP-HPLC were performed on grapes. Wines were made in triplicate from healthy, RB symptomatic, and second harvest RB symptomatic grapes, and analyzed for residual sugars, % EtOH v/v, volatile acidity, TA, free and bound $SO_2$, phenolic composition by RP-HPLC and protein precipitation and volatile composition by HS-SPME-GC-MS. Wine sensory characteristics were determined by descriptive analyses. Chemical analysis confirmed that grapes from symptomatic vines had decreased sugar and color accumulation (red varieties) and higher TA. Wines made from second harvest grapes from symptomatic vines showed less impact of the disease, producing wines with chemical, phenolic, and volatile profiles and sensory properties more similar to wines made from healthy fruit than wines made the first harvest of RB-diseased fruit.

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### Sources of Resistance to Root-Knot Nematode and Phylloxera

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A rootstock carrying resistance to a wide range of Phylloxera and root-knot nematode strains would be a useful tool to combat these soil-borne pests. The collapse of AxR1 in California identified a new biotype of Phylloxera, and root-knot nematode strains have overcome the resistance of Harmony and Freedom rootstocks.

## Viticulture — Disease Session – CONTINUED

The means by which these pests adapt to rootstocks is uncertain; however, the narrow genetic base of rootstocks may increase the chance of new, virulent pathotypes arising. The most commonly used rootstocks in California were developed ~100 years ago in Europe, and most have parentage that traces back to a single accession of *V. berlandieri*, *V. rupestris*, or *V. riparia.* There is a need to widen the genetic base of resistance by thoroughly examining wild *Vitis* germplasm and breeding rootstocks from novel forms of resistance. Some resistance is specific and only manifests during infection in the form of a hypersensitive reaction, thus preventing pest feeding completely. Other types provide tolerance to feeding, but are tolerant of a wide range of pathotypes. In this ongoing study, we are discovering new and specific resistance sources, focusing on specific resistances to develop new breeding lines, and developing mapping populations capable of identifying genomic regions associated with specific resistance to optimize rootstock breeding with DNA-based marker assisted selection.

*Funding Support: California Grape Rootstock Improvement Commission*



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## Enology — Wine Metals and Reactivity Session

### Rapid Determination of Active Sulfur Dioxide by Headspace Gas Chromatography

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Sulfur dioxide ($SO_2$) is perhaps the oldest and arguably the most important wine additive used in winemaking due to its antioxidant, antimicrobial, and enzyme-inhibiting properties. Conventional methods such as the Ripper titration, aeration-oxidation (A-O), and other analytical methods have been developed and employed widely for the quantitative analysis of $SO_2$ in wine. However, it is clear that a large fraction of the free $SO_2$ reported by these procedures is not actually available or "active" for protecting wine, due to the effects of weak binding with anthocyanins and other common compounds present in the wine matrix. A recently developed method to measure molecular and "free" $SO_2$ in wine using gas detection tubes (HS-GDT) demonstrated that levels of free $SO_2$ as determined by standard methods overestimate the free $SO_2$ in many wines. However, the gas detection tube method has not been widely adopted due to its complexity, especially for many samples. We describe an automated analytical strategy based on static headspace gas chromatography using sulfur chemiluminescence detection technology (HS-GC-SCD) to obtain what we define as the truly "active" free and molecular levels of $SO_2$ in wine. The HS-GC-SCD method proposed requires minimal sample preparation, can be automated, and can achieve results in as little as 10 min when the pH and ethanol concentrations are known. We will present information on a direct comparison of the Ripper, A-O, HS-GDT, and HS-GC-SCD methods on a diverse set of wine samples. Aside from a relatively high upfront cost for a GC system, the instrument's flexibility for other procedures, stability, and low operating costs per sample present opportunities for adoption by medium to large-sized operations. Additionally, direct measurement of "active" $SO_2$ may serve as a better predictor of wine aging and microbial stability and may be a useful tool for further research.

*Funding Support: American Vineyard Foundation, Henry A. Jastro Graduate Research Award*

### Fundamental Studies of Iron Oxidation Reactions in Wine-like Conditions

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Existing descriptions of the oxidation reactions in wine are independent of pH and the tartaric acid concentration. The iron-mediated catalysis of dioxygen to form reactive oxygen species constitutes the initiation step in the oxidation of wine. Over a broader pH range than that of wine, from pH 2.5 to 4.5, the speciation of Fe(II) and Fe(III) and their complexes change dramatically, therefore changing the rate at which these oxidation reactions can occur. In this experimental study, air-saturated model wine solutions of Fe(II) and tartaric acid were followed for the rates of oxygen consumption and Fe(III) formation. Attempts to measure hydrogen peroxide formation were also employed to assist in the elucidation of

the auto-oxidation nature of the kinetics. The auto-oxidation lag time and extent of oxygen consumption was pH dependent, while the maximum rate of oxygen consumption was pH independent. Additionally, the lag time, maximum rate of oxygen consumption, and extent of oxygen consumption were probed by adding varying amounts of reactants (Fe(II), tartaric acid), intermediates (hydrogen peroxide), and intermediate-scavenging agents (Cu(II), catalase, superoxide dismutase). Future work will attempt to express these oxidation curves using a pH- and metal speciation-based model.

*Funding Support: Stephen Sinclair Scott Endowment in Viticulture and Enology, Wine Spectator Fellowship, Treasury Wine Estates*

## Implementation of a Quick Test to Control Removal of Iron and Copper from Wine

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Iron and copper act as oxidation catalysts and can lead to browning reactions and other detrimental oxidative changes in wine. These compounds are introduced throughout the process from fungicides, fining agents like bentonite, and vineyard soil and winemaking material. Removal of heavy metals prior to bottling is critical to ensure prolonged shelf life and prevent accelerated aging. This goal can be achieved with the addition of phytic acid, potassium hexacyanoferrate(II) (PHCF), and a PVPP based synthetic polymer, Divergan HM. PHCF cannot legally be used in the United States and phytic acid only removes iron, so Divergan HM is currently the only approved method to remove copper from wine. In contrast to other fining methods, there is no inexpensive quick test for iron and copper before and after the application. The objective of this study was to implement a colorimetric quick test to control the removal efficiency of heavy metal fining agents in wine. The tests are based on complexation reactions of iron and copper with organic molecules and the comparison of the corresponding color on a scale in a range between 0.1 mg/L and 50 mg/L. Total iron can be analyzed by reducing $Fe^{3+}$ to $Fe^{2+}$ in the sample prior to the reaction. For the iron test, brown and red wines should be treated to remove color, while copper can be analyzed in any wine without pretreatment. The tests take about five minutes for iron and ten minutes for copper with a combined cost of $0.65 per sample. The test is a great alternative to lab-based methods, not only because of the low cost, but also because, being a quick test, it is easy to use in a winery environment, even by untrained personnel.

*Funding Support: N/A*

## Viticulture — General Viticulture Session

### Effect of Root Trimming and Planting Method on Early Vine Development

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Improper planting techniques that result in a distortion of the downward distribution of the roots of grapevine planting stock can increase the potential for poor growth and root disease. This upward curling of the root system is often referred to as "J" rooting. Reducing root length during planting may reduce the potential for "J" rooting. A field trial was established in a Pinot noir vineyard site west of Soledad, CA (Santa Lucia Highlands Viticultural Area), to evaluate (1) root trimming of dormant benchgraft roots to either 4 or 15 cm; and (2) planting method, by either digging a traditional hole or using a planting spade to dig a slot in which the benchgraft was inserted. The experimental design was a randomized complete block with 12 replications of the four treatment combinations. The bench grafts were planted in 2013 using SO4 rootstock and were trained as bilateral cordons on a vertically shoot-positioned trellis, then spur-pruned. Vine spacing was 1.8 × 1.8 m. Planting method and root length of dormant benchgrafts were observed to influence initial vine growth. Both trimming of roots to 4 cm and spade planting reduced vine growth in the first year. Of the two factors tested, only reducing root length resulted in less vine growth in the second year. In years three, four, and five, there were no significant differences in vine canopy growth as measured by pruning weight. Although there was some loss of initial vine growth by root trimming in the first two years after planting, initial yield in years three to five was not affected. Root trimming of dormant benchgrafts prior to planting could be an effective practice to reduce the incidence of "J" rooting, especially for high-risk methods such as spade planting.

*Funding Support: No external funding*

### Isohydric and Anisohydric Winegrape Varieties and Stomatal Response to Soil Water Availability

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Varieties of *Vitis vinifera* L. differ in their response to water availability. The attempt to classify varieties into the two categories of isohydric and anisohydric responses has produced much discrepancy among trials. We hypothesize that this is due to the lack of consideration for a biological continuum across varieties rather than the adoption of two extremes. Our goal was to study 18 *V. vinifera* varieties under the same environmental conditions. We imposed dry-down/recovery cycles and explored the behavior of the varieties over the full soil moisture range in 2015, 2016, and 2017. We used eight replicate vines per variety to measure midday leaf water potential and stomatal conductance, as well as soil moisture. Our results show that there may be three distinctive major patterns of midday leaf water potential response to soil water availability: linear drop across the entire soil moisture range, linear drop below a threshold of soil water content, and a plateau reached at a soil moisture threshold. Meanwhile, the stomatal sensitivity often did

not mirror the midday leaf water potential behavior; i.e., varieties with tight control of water status often had stomata insensitive to soil moisture depletion, while some varieties with a linear drop of water status had tight stomatal control. As transpiration is affected by vapor pressure deficit, some varieties showed a response to this parameter under high soil moisture, while others did not. These results may eventually be used by growers in irrigation management decisions.

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

## Changes In Splitting Susceptibility with Grape Variety and Berry Development

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Grape berry growth after veraison is regulated by internal pressure and the rheological properties of the skin. Internal pressure originating from cell turgor is the driving force for berry expansion. Skin elasticity, the combination of cuticle and a few cell layers, works against that internal pressure to constrain expansion. Berries may split if the skin accumulates excessive tensile stress. In this study, the mechanical response to internal pressure in berries from different cultivars was recorded using a custom-built injection tester. Based on the pressure-strain curve, the splitting resistance was determined. Further, the minimum internal pressure for berry growth and skin elasticity was estimated by determining yield strength. Compressive strain as an indicator of softness was also measured using a skin fold caliper. Hard-green Merlot, Syrah, and Zinfandel berries behaved as a brittle material, while soft berries had properties of viscoelastic material. In contrast, Concord grapes behaved as viscoelastic material even when the berries were still hard and green. The splitting resistance and yield strength generally correlated negatively with berry total soluble solids, except there was no correlation with yield strength in Concord. Compressive strain increased abruptly in berries with less than 10 Brix and in ripe berries. Since splitting resistance and yield strength were relatively stable during ripening, the abrupt increase of compressive strain suggested that internal pressure decreased due to dehydration in overripe berries. This study suggests that the minimum turgor for berry growth decreased at the onset of ripening and the responses of splitting resistance and yield strength to internal pressure remained unchanged by late-season berry dehydration. Thus, grape berries are susceptible to splitting after the onset of softening and before being fully ripe.

*Funding Support: Chateau Ste. Michelle Distinguished Professorship, Washington State Grape and Wine Research Program, Washington State University Graduate School*



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## Enology — General Enology Session

### Comparing Nitrogen Addition in the Vineyard versus in the Winery on Pinot noir and Chardonnay

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The impact of nitrogen (N) additions in the vineyard on vine productivity and wine characteristics was compared to that of winery N additions on wine characteristics in Pinot noir (PN) and Chardonnay (CH). Five treatments, including no vineyard or winery N addition (control), addition of diammonium phosphate in the winery (+DAP), addition of organic N in the winery (+Org N), and addition of N in the vineyard to the soil (+Soil N) or to the foliage (+Foliar N) were established with four replicates for each variety. The +Foliar N treatment began in 2017, while the other four treatments were evaluated in 2016 and 2017 for CH. For PN, all five treatments began in 2017. In both varieties, +Soil N application increased leaf N status and fruit yeast assimilable nitrogen (YAN) levels, but did not alter yield, leaf area, or vine water status in the first year. Foliar N application also increased fruit YAN in the first year without increasing leaf N or vine growth. Vine growth and yield increased in CH in response to +Soil N in the second year, as did leaf and fruit N status. Root colonization by arbuscular mycorrhizal fungi (AMF) was reduced in both varieties in the +Soil N vines, with greater effect in CH, where N was applied for two years. Vine P status decreased in CH in the second year in concert with lower AMF. Winery N additions boosted must YAN levels to roughly match those of the +Soil N and +Foliar N musts. Must fermentation in CH proceeded more rapidly where N was added in the vineyard or the winery, while fermentation rate in PN was increased only by vineyard N addition. Sensory evaluation of 2016 CH wines showed that +Soil N wines differed from control, +DAP, and +Org N wines.

*Funding Support: Oregon Wine Board and USDA-ARS*

### Polyfunctional Thiols in Wine: Chirality, Precursor Stereochemistry, and Sauvignon blanc Clone Type

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Impact odorants that significantly influence aromas of fragrances, foods, and beverages are of particular scientific interest due to their presence across different commodities and their high potency. One class of well-known impact odorants that impart "tropical" aromas to wine is the polyfunctional thiols, including 3-sulfanylhexan-1-ol (3-SH) and 3-sulfanylhexyl acetate (3-SHA). Since their first identification in wine around two decades ago, 3-SH and 3-SHA, also known as varietal thiols, have been the focus of ongoing investigations due to their extremely low sensory thresholds (ng/L) and aroma qualities reminiscent of "passionfruit" and "grapefruit." Following our previous studies into 3-SH and 3-SHA in particular, we have now applied chemical synthesis, developed and validated new mass spectrometry-based stable isotope dilution analysis (SIDA)

methods, used a high-throughput fermentation robot, and undertook studies to explore the enantiomeric distribution of 3-SH and 3-SHA in wines arising from five Sauvignon blanc clones. The new analytical methods were also applied to a range of commercial wines to investigate the chiral distribution of varietal thiols and the potential fate of 3-SH in wine. The fermentation trials provided insight into the stereochemical relationship between 3-SH and 3-SHA and their diastereomeric precursors present in the juice, providing a better understanding of the formation and fate of polyfunctional thiols in wines.

*Funding Support: Wine Australia*

## Optimization of Winery Cleaning and Sanitation: Effective Chemistries for Microbial Inactivation and Fermentation Soils

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Cleaning and sanitizing are essential aspects of wine production. Winery spoilage microbes can exist in planktonic physiologies or as biofilms, making the organisms variably resistant to antimicrobial agents. Commonly used winery cleaning and sanitizing chemistries were screened for effective inactivation of seven winery spoilage organisms (*Acetobacter pasteurianus, Lactobacillus casei, Oenococcus oeni, Pediococcus parvulus, Brettanomyces bruxellensis, Saccharomyces cerevisiae,* and *Zygosaccharomyces bailii)* in 96-well microtiter plates. Effective cleaners were determined via plating for planktonic treatments and with crystal violet staining for biofilm biomass remaining in the microtiter plates. The most effective treatments from these trials were used to analyze the inactivation and removal of biofilms grown on 2B mill stainless steel (SS) coupons. Biofilms remaining on the coupons were measured using ATP swabs and tip plating for enumeration of viable colonies. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined for treatments that were effective in the coupon trials. Live/dead fluorescence staining was used to analyze minimum effective contact times. These results were used in red wine fermentation trials to create an optimized SOP for SS cleaning. Caustic-based cleaning agents are not only most effective at removing biofilms and soil in well plates and SS, but also provide high levels of microbial inactivation and serve as dual cleaner/sanitizers. Biofilm communities were not effectively removed from SS by agents marketed as sanitizers, suggesting a cleaning step is necessary for surfaces, even in visually clean states. MBC and MIC values varied among organisms and are lower than manufacturer's recommended levels for caustic treatments. Fluorescence staining was a useful tool to determine viability of microbe populations and for observing biofilms with confocal microscopy.

*Funding Support: American Viticulture Foundation*



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## Viticulture — Crop Load Management Session

### Crop Load Management of Newly Planted Pinot gris in the San Joaquin Valley of California

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San Joaquin Valley (SJV) has 65% Pinot gris acreage and the majority of Pinot gris crush volume (83%) in California. Strong demand for Pinot gris has prompted growers to restrict the nonbearing period to <2 years, if possible. This requires permanent vine structure establishment the first year, with a crop expected in the second year. Precocious cropping raises the risk of overcropping, with possible carryover effects in subsequent years. To identify the optimum crop level and economic threshold for newly planted Pinot gris vines, a trial was initiated in a commercial vineyard in 2016. Four crop levels, replicated five times, were established three weeks before bloom; 0 (defruited), ½ (one cluster per two shoots), one cluster per shoot, and unthinned vines (no clusters removed). Cluster removal increased fruit set, average berry weight, and soluble solids. Increased cluster compaction on thinned vines did not cause excessive bunch rot, but did partially compensate for the potential yield loss associated with cluster removal. Yield in 2016 was reduced by 6, 28, and 100% with the severity of cluster removal. No thinning was performed in 2017, but yield and pruning weight were measured. The Ravaz index (RI) of vines with ½ cluster per shoot was 8.3 in 2016, and vines in that treatment had the highest accumulated yield across 2016 and 2017. Vines with RI > 10 showed significantly delayed ripening in 2016 and reduced yield in 2017. Thus, young vines with an RI > 10 in their first crop year were overcropped and will likely have reduced yields the following year, while vines with an RI of 10 provided maximum yield without affecting fruit quality and the following year's crop. The study is ongoing to determine the duration that overcropping in the first year may affect the vines.

*Funding Support: University of California Agriculture and Natural Resources and The Wine Group*

### Pinot noir Yield Potential and Nutrient Reserves under Long-Term Vineyard Floor Management

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Pinot noir has lower yield potential than many winegrape cultivars and annual yield variation is considerable in the cool climate of western Oregon. Climate and nutrient status may influence yield, but information on their physiological impacts on yield predictors such as fruitfulness is lacking. High vegetative vigor and shading have been related to poor bud fruitfulness; many vineyards in the region are characterized by high vegetative growth that requires canopy management to ensure adequate light infiltration. A long-term vineyard floor management trial was established to alter Pinot noir vine vigor using perennial grass cover (Grass), tillage (Tilled), or a combination of both (Alternate) in alleys flanking vine rows. Yield and vine size were altered by years four to six. It was hypothesized that lower

yields were a result of both low vine nitrogen (N) and carbohydrate reserves by way of reduced bud fruitfulness. During years seven through nine, the trial was used to determine whether vine N and carbohydrate status influenced the presence of floral primordia in buds, fruitfulness, and yield at harvest. Grass vines had reduced veraison leaf area, pruning weight, and yields compared to Tilled vines, and the effect was likely due to the lower N status of Grass vines. Yield reduction was due to reduced fruitfulness that related to low vine N status, rather than to the vine total non-structural carbohydrate status (TNC). Grass vines had 0.2 fewer inflorescences per bud than Tilled, which reduced yield by ~43% in two of three years. Although Grass vines had higher canopy light infiltration than Tilled, buds were less fruitful. This study suggests that vine N status of both annual tissues and reserves is a more important determinant of yield capacity than light exposure, photoassimilation, and TNC status.

*Funding Support: Oregon Wine Board Agriculture Research Foundation*

## Long-Term Weather Variability and Concord Grape Berry Weight Dynamics

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Weather plays a key role in growth and development of Concord (*Vitis labruscana* Bailey). Short-term weather variability can lead to variation in annual Concord yield and influence the market. Concord growers need to precisely estimate yield early in the season to make better management decisions such as shoot or fruit thinning. Berry weight is one of the key components for Concord crop estimation. The main objective of this study was to investigate any potential correlation between seasonal weather patterns and berry weight dynamics across multiple growing seasons. Long-term historic weather data were obtained for Lake Erie, NY, and the temperature and precipitation for each growing season was classified into normal, below-normal, or above-normal. Berry weight data were collected on a weekly interval at the Cornell Lake Erie Experimental Vineyard, starting 20 days after bloom until harvest for 17 consecutive growing seasons. At harvest, juice soluble solids, pH, titratable acidity, and color of berry samples were also measured. The collected data were then analyzed based on growing season classifications. This classification was later used to estimate berry weight, the results were compared to the observed berry weight, and the bias was computed. In years with a cold and wet July, the average berry weight tended to be higher (3.44 g versus 2.76 g) than in years with a warm and wet July. A strong correlation was found between berry color and maximum temperature (r = 0.7) and rain (r = -0.71) in July. The mean absolute bias averaged 0.24 g and RMSE was 0.25 when the estimated and observed berry weights were compared. This study described the trend between weather variability and berry weight dynamics across multiple growing seasons.

*Funding Support: National Institute of Food and Agriculture*



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## Enology — Microbiology Session

### Impact of Non-*Saccharomyces* Yeast on *Hanseniaspora uvarum* Growth and Volatile Acidity Production during Cold Soak

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*Hanseniaspora uvarum* is often the most abundant yeast present on grapes at harvest and is a primary source of volatile acidity during cold soak. *H. uvarum* growth during cold soak is typically controlled using sulfur dioxide and by maintaining grapes at a cold temperature. While effective, these methods can also restrict the growth of other, non-*Saccharomyces* yeast present during cold soak that can contribute positively to wine aroma. Recently, addition of select non-*Saccharomyces* yeast at the beginning of cold soak has been suggested as an alternative method to reduce volatile acidity. This study investigated the efficacy of select non-*Saccharomyces* yeast strains to reduce volatile acidity and *H. uvarum* growth during cold soak. Commercially available non-*Saccharomyces* yeasts were screened for their ability to reduce *H. uvarum* growth and acetic acid production during a simulated cold soak in a grape juice-based medium. *H. uvarum* growth and acetic acid production was reduced in the presence of all non-*Saccharomyces* yeast tested, with some yeast having a greater impact than others. One yeast, *Metschnikowia fructicola*, was then used in Pinot noir winemaking experiments. Pinot noir grapes were inoculated with a combination of *H. uvarum* and *M. fructicola,* then cold-soaked for six days. During cold soak, *M. fructicola* reduced *H. uvarum* growth and significantly decreased acetic acid and ethyl acetate production. These results suggest that adding non-*Saccharomyces* yeast during cold soak may be an effective method to reduce the production of volatile acidity by *H. uvarum*. Additional work is being conducted to test the effecacy of these cultures against other *H. uvarum* strains.

*Funding Support: Oregon Wine Board*

### Butter Bomb or Fruit-Driven Chardonnay – How Genetics and Metabolomics Can Assist with the Decision

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Diacetyl is a major flavor metabolite produced by wine-associated lactic acid bacteria (LAB). In wine, diacetyl has important stylistic implications. Depending on the style and type of wine, it is considered to contribute a desirable "buttery" or "butterscotch" flavor character. For a fruit-driven white wine, diacetyl production should be minimized, because it will mask the varietal aroma. Formation of diacetyl is closely linked to the growth of wine LAB such as *Oenococcus oeni* and the metabolism of sugar, malic acid, and citric acid. As part of the selection and commercialization process, potential starter strains are studied for their citric acid metabolism and diacetyl formation. A study undertaken by Bartowsky et al. (2010) in Cabernet Sauvignon wines demonstrated that different *O. oeni* strains will produce significantly different concentrations of diacetyl when used in

sequential MLF inoculation. Recently, Mink et al. demonstrated that pyruvate is a central metabolite in diacetyl synthesis by *O. oeni*. Diacetyl formation by *O. oeni* is induced by yeast-derived pyruvate in the early stage of winemaking, resulting in diacetyl accumulation in the wine. We have analyzed and reassembled 296 *O. oeni* genome sequences, including those publicly available and from the University of Bordeaux. The citrate operon of a group of *O. oeni* strains and citric acid metabolism was examined to identify potential genetic markers that could predict the consumption of citrate during MLF. Besides choosing the appropriate strain, application of certain winemaking practices will also help modulate diacetyl content in wine through malolactic fermentation.

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## Building a Genome-Scale Mathematical Model for Yeast to Understand Differences in Metabolism among Commercial Strains

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Two key metabolic activities relevant to industrial wine fermentations are nutrient use efficiency and tolerance to high ethanol concentrations exhibited by industrial yeast strains. The details of yeast metabolism is of great interest to develop ways to control stuck or sluggish fermentations. One approach is to use computational methods, due to their advantage of being comprehensive and more economic than experimental methods. Hence, many studies have been conducted to create genome-scale metabolic models of yeast. Despite progress in the field, most current models either focus on aerobic systems or lack the detailed lipid metabolism that has been shown experimentally to be highly correlated with nutrient use efficiency. One way to capture the power of these models is to use dynamic FBA (flux balance analysis) to predict the flux distribution of all metabolites within the cell over the course of an entire fermentation. Using this approach, it is possible to test the predictive capability of these models by comparing predictions with experimental fermentation data. Once the models fit dynamic data, they can be used to understand differences among commercial strains and suggest genetic modification strategies to increase strain ethanol tolerance and nutrient use efficiency. In this study, we improve the latest consensus genome scale model of yeast by incorporating additional lipid pathways. Previously, we showed that nutrient use efficiency and ethanol tolerance of 22 different industrial yeast strains were a strong function their lipid composition, while molecular mechanisms of these phenomena were not elucidated. By using the Yeast 7.6 model, which has the most comprehensive representation of fatty acid, glycerolipid, and glycerophospholipid metabolism, we can more accurately predict metabolic fluxes for various yeast strains and understand the variation in metabolism among different strains that leads to disparities in nutrient use efficiency and aroma production.

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## Enology — Microbiology Session – CONTINUED

### The Estimation of Fermentation Parameters in Research and Commercial Winemaking

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Mathematical models for wine fermentations and parameter estimation for algorithms have been available for over 35 years; however, only recently, with advances in data collection systems and accurate methods for automated Brix measurements, have these fermentation parameters found meaning in commercial winemaking. Using the Boulton fermentation model and Bard parameter estimation algorithm, on-line Brix curves from more than 100 research-scale and commercial fermentations were analyzed numerically for the initial lag period, the initial nitrogen concentration, the specific maintenance rate of the yeast, and the viability constant of the yeast population. These fermentation parameters and their ranges are reported and interpreted in the context of distinguishing complete from stuck fermentations. Examples of early fermentation modeling combined with predictive forecasting based on these parameters that would allow winemakers to consider early intervention to avoid stuck fermentations are presented. Such modeling also provides the near-future cooling requirements that can be used in the management of refrigeration loads and integrated energy systems.

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## Viticulture — Viticulture from East to West Session

### Sour Grapes, Indeed! Malic Acid Increases in Certain *Vitis* spp. during Maturation

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Wild *Vitis* and their interspecific hybrids have very high malic acid concentrations (10 g/kg or more), even at late harvest dates, and high soluble solids (>20 Brix). The behavior of malic acid in non-*vinifera Vitis* spp. during berry maturation would be of interest to grape breeding programs and viticulturalists, but to our knowledge, has not been reported. Over a two-year study, multiple accessions of *V. riparia* and *V. cinerea* were collected from the USDA ARS Cold Hardy Grape Germplasm Collection (Geneva, NY) and *V. vinifera* and interspecific hybrid grapes were collected from nearby commercial vineyards. Sampling was performed at three to four time points from preveraison to typical commercial sugar maturity (>20 Brix). We observed no significant difference in malic acid among *riparia*, *vinifera*, or interspecific hybrids preveraison; however, malic acid (on a g per berry basis) decreased more slowly in *riparia* than in *vinifera*, with intermediate degradation rates observed in interspecific hybrids. In some *riparia* accessions, we observed no significant change in malic acid per berry between the preveraison maximum and harvest. More surprisingly, malic acid increased in *cinerea* accessions during berry ripening. These findings suggest that high levels of malic acid in wild *Vitis* and interspecific hybrids as compared to *vinifera* result from much lower rates of malic acid degradation and/or continued malic acid synthesis during ripening.

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### Influence of Plant Growth Regulators on Autumn King Table Grapes under Two Training Systems

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Autumn King is a late-season, white seedless table grape developed by David Ramming and Ronald Tarailo of the USDA-ARS in Fresno, CA. This variety naturally produces large berries, but gibberellic acid ($GA_3$) and forchlorfenuron (CPPU) can be used to manipulate berry size and cluster quality. However, there is a lack of information on the optimum $GA_3$ and CPPU treatments for Autumn King. Moreover, Autumn King may be spur- or cane-pruned, with possible effects on fruit quality. Therefore, studies were conducted in a commercial Kern County table grape vineyard with mature, uniform, own-rooted Autumn King grapevines with two different training systems: head-trained, cane-pruned, or quadrilateral cordon-trained, spur-pruned vines. Vines were subjected to one of four different $GA_3$ thinning sprays (0, 0.5, 1, or 2 ppm GA3) in combination with varying treatments of sizing using $GA_3$ at 2 or 10 ppm and CPPU at 2 or 6 ppm. We found that all of the bloom $GA_3$ treatments reduced the number of berries per cluster on spur-pruned vines, but 1 ppm $GA_3$, was needed to adequately thin cane-pruned



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vines. The greatest cluster and berry weight, size, and Brix were obtained using 1 ppm GA at bloom, followed by GA at 10 ppm or CPPU at 6 ppm. No treatment combination caused excessive cluster compactness or preharvest bunch rot. Our data showed that none of the treatments had any negative effect on vine fruitfulness during the last two seasons. Autumn King is a protected variety, and the California table grape commission is the exclusive licensee.

*Funding Support: California Table Grape Commission*

### Sunpreme, a Raisin Grape with Novel Traits, on Different Rootstocks and Trellises

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Sunpreme, a new USDA raisin grape variety, has two key traits: fruitful basal buds and fruit that dries spontaneously after ripening. These unique traits should make it possible to prune vines and harvest raisins with machines, with little, if any, additional labor needed for either practice. Complete mechanization would be a revolutionary change for raisin production practices, which historically have been among the most laborious of any horticultural crop. Basic insight on how cultural practices may affect the viticultural performance of Sunpreme, including possible effects of rootstock and trellising, are lacking, but necessary to fully capitalize on this variety's unique traits. It is also necessary to better understand one of Sunpreme's undesirable traits, preharvest fruit drop, as this problem could limit yields and possibly complicate mechanical harvest operations. Therefore, we established a Sunpreme vineyard at the Kearney Agricultural Center in Parlier, CA, in a split, split plot design, where the main plot factor was trellis (single high wire or 0.8-m wide T), the subplot factor was rootstock (None, Freedom, or 1103P), and the sub-subplot was thinning (0 or 40% cluster removal at fruit set). Vines on T-trellises were more productive than vines on bilateral cordons, due to differences in the number of shoots per vine. Rootstocks strongly affected vine mineral nutrient status, but had little effect on productivity except that the fruit from vines on rootstocks appeared to mature slightly earlier than those on own-rooted vines, resulting in drier raisins at harvest. Thinning increased berry weight, decreased yield, and increased bunch rot. Preharvest fruit drop reduced potential yield by ~10%, and 5 to 10% of the remaining raisins were lost during machine or hand harvest, respectively. Observations on the cause of fruit drop will be illustrated and discussed.

*Funding Support: California Raisin Marketing Board*

### Grape Ripening Control through Source–Sink Ratio Manipulation

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Canopy management and fruit load control seek to maintain a balance between a vine's sources and sinks. In fact, balanced vines may produce more consistent

yields and ripen more evenly. This study aims to study the relationship between source–sink ratios and important parameters for production logistics and grape quality, such as progress of ripening and grape composition at harvest. After homogenizing all vines (*Vitis vinifera* cv. Cabernet Sauvignon) by removing laterals and adjusting the number of shoots to 20, we tested three levels of canopy density and fruit load combined in a factorial design (3 × 3). Three canopy levels with 100, 66, or 33% of the leaves were combined with three fruit loads, 100, 66, or 33% of the fruit, corresponding to 30, 20, or 10 clusters per vine, respectively. Carbon fixation rates were transiently higher in plants with 33% of the canopy mediated by higher chlorophyll content, although this did not compensate for their smaller leaf area. The onset of ripening was sequentially delayed in 66% and 33% canopy treatments. The progress of ripening, accumulation of soluble solids, and loss of acidity (increase in pH and decrease in total acidity) was also slower in 66% and 33% canopy treatments than in vines with 100% of the canopy. The time to reach commercial maturity (>25 Brix) was delayed six weeks for the 33% canopy level. Surprisingly, fruit load did not have a significant effect on the progress of ripening. When comparing all treatments at commercial maturity, the treatment maintaining 100% of the canopy had the highest total acidity and lowest pH. The anthocyanin content was slightly lower in this treatment. These results provide a basis to control the speed of ripening, aiming to coalesce variability within a vineyard or optimize the tank capacity through sequential ripening.

*Funding Support: Oakville Experimental Vineyard*



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## Enology and Viticulture

### Pinot noir Hydroxycinnamic Acid Content under Different Aging Conditions and Volatile Phenol Production by *Brettanomyces*

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The volatile phenol precursors *p*-coumaric and ferulic acid, naturally present in grapes, are typically found as esters of tartaric acid (coutaric and fetaric acid, respectively). The esterified forms, often present in higher concentrations in wine than the free forms, can be hydrolyzed during the winemaking process. *Brettanomyces bruxellensis* can use the hydrolyzed, free form of these hydroxycinamic acid esters to create volatile phenols but cannot degrade the esterified forms. Therefore, this study investigated factors that may impact the hydrolysis of esterified hydroxycinnamic acids during winemaking, including malolactic fermentation (MLF), wine pH, wine ethanol content, and wine storage temperature. Pinot noir wines were produced and MLF was conducted with either a cinnamoyl esterase-positive or cinnamoyl esterase-negative *Oenococcus oeni* strain. At the completion of MLF, there were significantly higher concentrations of *p*-coumaric acid in wines where MLF was performed by the cinnamoyl esterase (+) *O. oeni* strain than in wines where the cinnamoyl esterase (-) strain was used. Wines were then adjusted to two different pH values and two different ethanol concentrations, sterile-filtered, bottled, and stored at either 13 or 21°C. Wines were assessed for esterified and free hydroxycinnamic acids after 0, 30, 100, and 180 days in storage. The concentrations of esterified and free hydroxycinnamic acids remained constant throughout aging. Concentration differences seen at the end of MLF remained after aging 180 days, regardless of wine pH, ethanol, or temperature of storage. At the end of aging, wines were inoculated with *B. bruxellensis,* growth was monitored for sixty days, and the wines were assessed for volatile phenol concentrations.

*Funding Support: Northwest Center for Small Fruits Research*

### A Method Based on Loop-Mediated Isothermal Amplification Was Developed to Detect *Brettanomyces bruxellensis* Rapidly

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*Brettanomyces bruxellensis* is one of the major spoilage microorganisms in wines, responsible for off-odor. In recent years, *B. bruxellensis* contamination has become more common in China. Conventional wine treatments will not prevent contamination, partially due to its remarkable $SO_2$ and ethanol tolerance compared with *Saccharomyces cerevisiae*. Thus, it is critical to detect it early during winemaking. Traditional methods such as differential medium or specific PCR amplification can detect *B. bruxellensis*, but such methods need time, trained technicians, and specialized equipment that may not be available to a winery. Therefore, a simple and quick way to detect the presence of *B. bruxellensis* in wine is desirable. We have developed a method to detect *B. bruxellensis* rapidly, based on loop-mediated isothermal amplification. The primers were designed to target on the sequence of 5.8S-ITS-18S and specifically amplify the DNA genome of *B. bruxellensis*. The

method targets six specific regions, designed inside and outside of four primers by design software. The LAMP reaction system was optimized for dNTP concentration, $Mg^{2+}$ concentration, and reaction temperature. The results showed that 0.8 mmol/L dNTP, 3.0 mmol/L $Mg^{2+}$, and a reaction temperature of 62°C is suitable. In addition, we adopted visual observation and analysis to verify whether a reaction occurs by white precipitate or fluorescence staining. The method was specific and could verify the presence of several known yeasts. The detection threshold of DNA was down to 15 pg/μL. Compared with traditional PCR, LAMP is simple to operate and low-cost. It does not rely on any special instrument or equipment to detect *B. bruxellensis* rapidly under practical production conditions.

*Funding Support: Northwest A&F University, China*

## Yeast Species Associated with Texas High Plains Vineyards and Natural Fermentations of Tempranillo Grapes

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The main yeast species present on grapes, leaves, and surrounding soils of Tempranillo and Cabernet Sauvignon vineyards in the hot, semi-arid climate of the Texas High Plains area were investigated, as well as the presence and evolution of yeast species during natural fermentations of Tempranillo grapes from the same vineyards. Characterization of yeast species was performed using the culture-dependent ITS-RFLP method and genome sequencing. Yeast species recovered from grapes, leaves, and soils were mainly dominated by *Aureobasidium pullulans*, *Cryptococcus* species, *Filobasidium* species, and *Naganishia* species, typical members of the vineyard environment. Only one isolate of potential enological interest, *Lachancea thermotolerans*, was recovered from the vineyard environment. However, natural fermentation revealed the presence of the fermenting yeast *Saccharomyces cerevisiae, L. thermotolerans,* and *Hanseniaspora species*. The presence of *L. thermotolerans* is of extreme interest for winemaking in the Texas High Plains. Indeed, this species has been previously shown to acidify musts, and one of the main characteristics of grapes grown in the Texas High Plains is their lack of acidity. These findings agree with the theory that microorganisms present in the vineyards of a specific area could be more adapted for fermentation of grapes grown in the same area, and could lead to the production of more typical and natural wines.

*Funding Support: Texas Tech University*

## A Two-Year Survey of Pinot noir Vineyard-Associated *Saccharomyces* Populations in the Okanagan Valley

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The yeast species *Saccharomyces* is present in vineyards and wineries and is responsible for the fermentation of grape must into wine. The potential contribution of

*Saccharomyces* yeasts to regional characteristics of wine, or terroir, is gaining attention. Studies suggest that there are unique subpopulations of *Saccharomyces* strains in various winemaking regions, but Canadian vineyard-associated *Saccharomyces* populations have not been regionally profiled. This two-year study characterizes the *Saccharomyces* populations in Pinot noir vineyards of British Columbia's Okanagan Valley (OKV), one of the major winemaking regions in Canada. Pinot noir grapes were collected in the 2016 and 2017 vintages from 13 vineyards across three OKV subregions spanning ~100 km (Oliver-Osoyoos, Penticton-Naramata, and Kelowna) and fermented in the lab to enrich for *Saccharomyces* yeasts. Among different subregions, there was a high disparity in spontaneous fermentation success rate over both vintages. In the 2016 and 2017 vintages, 1632 and 1440 *Saccharomyces* yeasts were isolated, respectively. To genetically characterize *S. cerevisiae* strains, microsatellite analysis was performed on all isolates by multiplex PCR of 11 genomic loci. Commercial *S. cerevisiae* strains were identified by comparing the microsatellite profiles to our commercial *S. cerevisiae* strain database of over 250 strains. *Saccharomyces* species and strain composition varied across subregions and vintages. In particular, *S. cerevisiae* was isolated in 2016, while *S. cerevisiae* and *S. uvarum* were isolated in 2017. Phylogenetic analysis suggests that potentially indigenous (noncommercial) sub-populations of *S. cerevisiae* are present in the OKV. Our long-term goal is to characterize the vineyard-associated *Saccharomyces* strains in the OKV and develop fermentation starter cultures that can produce wine with regional characteristics in a predictable and effective way.

*Funding Support: British Columbia Wine Grape Council and Mitacs Accelerate*

## Alcohol and Ester Evolution during Red and White Wine Fermentations

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Higher alcohols and esters are produced during wine fermentations. These compounds are fundamental to wine aroma. Some studies have shown that they could also be involved in headaches that occur after wine consumption. Our study aimed to determine the evolution of esters and higher alcohols during red and white wine fermentations. Red fermentations from Cabernet Sauvignon grapes were carried out at room temperature with three different yeast strains: BM45, EC 1118, and ICVD 254. Initial Brix was 20, 22, 24, and 26 for strain BM45, while an initial Brix of 26 was used for both EC1118 and ICVD 254. White fermentations were carried out at room temperature from Chardonnay juice. BM45, Rhone 2056, and Rhone 2226 yeast strains were used, with initial Brix of 20, 23, and 26. Esters and higher alcohols were quantified by GC-MS during fermentation. Samples were centrifuged for 10 min at 5000 rpm, filtered through a 0.45µm PTFE, and then analyzed. Five compounds were observed to evolve significantly during fermentation of both white and red wines: 2-methyl-1-propanol, 1-propanol, 2 methyl-1-butanol, phenyl ethyl alcohol, and ethyl acetate. For all the compounds, concentrations increased initially during the fermentation and then reached a plateau before fermentation was complete. The highest concentrations were noted for 2-methyl-1-butanol in both the red and white fermentations, while the lowest

levels were observed for 2-methyl-1-propanol in the red wines and ethyl acetate in the whites. The initial sugar concentration correlated with the concentrations of only 1-propanol and ethyl acetate. In addition, yeast strain had an impact on the production of higher alcohols and esters.

*Funding Support: UC Davis*

### Accomplishing Spontaneous Chardonnay Fermentations in the Okanagan Using the Alternative Yeast *Saccharomyces uvarum*

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Most wines are produced by inoculated fermentations using known, commercialized strains of *Saccharomyces cerevisiae,* but there is a growing trend in winemaking of performing spontaneous fermentations, which rely on microflora present on grape berries or winery equipment. An advantage for spontaneous over inoculated fermentation includes a more complex sensory profile due to a wider range of metabolites being produced by differing yeast species, which may help to define the microbial terroir of the wine. Spontaneous fermentations are characterized initially by a diversity of yeasts coming from the winery and vineyard environments, but strains of ethanol-tolerant *S. cerevisiae* eventually dominate the end of fermentation. However, previous studies in our lab identified *Saccharomyces uvarum* (a non-inoculated yeast) in spontaneous Chardonnay winery fermentations of grapes from the Okanagan Valley in British Columbia as the dominate yeast over *S. cerevisiae* during all later stages of fermentation. Although the overall objective of this study was to determine the origin (winery or vineyard) of Okanagan *S. uvarum*, we report here on the abundance of *S. uvarum* in relation to *S. cerevisiae* and the *S. uvarum* strain diversity of 2017 spontaneous Chardonnay fermentations coming from two different Okanagan vineyards. Using culture-dependent methods, we identified nearly 1000 isolates from winery fermentations and discovered that *S. uvarum* was again dominant over *S. cerevisiae* in all fermentations, as in 2015 in the same winery. We also report strain results from an *S. uvarum* 11-loci microsatellite multiplex screening. Overall, our results indicate there may be commercial interest in using *S. uvarum* as a potential alternative fermenting yeast to *S. cerevisiae*.

*Funding Support: Natural Sciences and Engineering Research Council of Canada - Collaborative Research and Development Grant (NSERC-CRD)*

### Identification and Characterization of Non-*Saccharomyces* Yeasts Isolated from British Columbian Vineyards

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Spontaneous fermentations are used in the boutique wine industry despite the unpredictable nature of this method compared to inoculated fermentations using single strains of the yeast *Saccharomyces cerevisiae*. Wines made by spontaneous fermentation may have enhanced aroma and flavor complexity due to the impact



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of secondary metabolites produced by various non-*Saccharomyces* (NS) yeasts present on wine grape skins and in the winery environment. Wine and vineyard-associated microbial communities vary in composition by region and are influenced by factors such as climate and viticultural practices, all of which contribute to the unique character or terroir of wines produced by spontaneous fermentation. The Okanagan Valley in British Columbia, Canada, is home to over 130 wineries, many of which favor the spontaneous fermentation method as part of their wine portfolio to capitalize on the effects of this unique microbial terroir. The purpose of this study is to identify the NS yeasts isolated from 13 Okanagan Valley vineyards and to evaluate their performance in single and sequentially inoculated fermentations. Pinot noir grape clusters from the Oliver-Osoyoos, Penticton-Naramata, and Kelowna wine subregions were sampled in 2016. Grape samples were spontaneously fermented in the lab and yeast were isolated at various fermentation stages. ITS and D1/D2 28S rDNA Sanger sequencing identified 18 yeast species from 2016, with eight additional species identified from a single Oliver-Osoyoos winery sampled in 2015. Ten NS yeasts were evaluated for fermentation ability by inoculation into Chardonnay grape must, followed by metabolite analyses after 28 days. Sequential fermentations were also conducted with *S. cerevisiae* and NS yeasts with the most promising fermentative performance and metabolite profiles. These results will help elucidate the unique behavior of indigenous yeasts from the Okanagan Valley that may be candidates for use in commercial-scale sequentially inoculated fermentations.

*Funding Support: NSERC Discovery Grant, BC Wine Grape Council*

## A Tale of Two Wineries: How Minimal Intervention Techniques Can Add Diversity to the Conventional Cellar

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In modern-day winemaking, producing consistent wines year after year is often desirable. To achieve this, many winemakers add sulfur dioxide ($SO_2$) and commercial yeasts to their fermentations to prevent the growth of unwanted microbes and ensure that fermentation will complete without undesirable by-products. However, there has been a shift in recent years, partially driven by consumer preferences, for wines produced with minimal input by the winemaker, for a more true expression of the grapes and the wine region they're grown in. To investigate the implications of this shift, experimental fermentations were conducted at two wineries in the Okanagan Valley to assess the potential for introducing minimal intervention techniques into conventional cellars. At the first winery in 2014, we studied how different levels of $SO_2$ at crush (0, 20, and 40 mg/L) can alter which yeasts conduct uninoculated fermentations. All treatments were dominated by a high diversity of commercial *Saccharomyces cerevisiae* strains, and each treatment had a significantly different strain assemblage, resulting in measured differences in wine sensory profiles. At the second winery in 2015, a *pied de cuve* inoculation factor was added, where vineyard-specific yeasts were used to initiate fermentation. The effect of two levels of $SO_2$ added at crush (0 and 40 mg/L) and two types of fermentation

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(uninoculated and *pied de cuve* fermentations) were compared. All four treatments were dominated by a diversity of indigenous *Saccharomyces uvarum* strains, and fermentations with different $SO_2$ treatments contained significantly different strain assemblages as well as unique wine sensory profiles. *Pied de cuve* inoculation did not significantly impact the fermentation or final wine. These results are of particular interest to winemakers looking to introduce minimal intervention techniques into their winemaking practices, while mitigating the risks associated with uninoculated and unsulfited fermentations.

*Funding Support: Natural Sciences and Engineering Research Council of Canada; American Society for Enology and Viticulture; The British Columbia Wine Grape Council*

### Microbiological Characteristics of Sulfite-Free Wine and the Development of a Detection Medium for Acetic Acid Bacteria

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Owing to an increasing consumer preference for additive-free food, the sulfite-free wine industry has been expanding in Japan. Sulfite-free wine generally exhibits decreased resistance to spoilage microbes such as yeast, lactic acid bacteria, and acetic acid bacteria. Therefore, it is necessary to conduct a more rigorous microbial quality control of sulfite-free wine than of wine containing sulfite. In this study, we specifically examined the thermal resistance of lactic acid bacteria and acetic acid bacteria in phosphate-buffered saline containing ethanol. Lactic acid bacteria showed an approximately three-log reduction after heating at 55°C for 3 min, while acetic acid bacteria showed more than a five-log reduction after heating at 50°C for 3 min. Next, we developed a detection medium for wine spoilage acetic acid bacteria such as *Acetobacter pasteurianus*. The optimal growth conditions for *A. pasteurianus* strains in yeast peptone dextrose (YPD)-based medium supplemented with various components were investigated. Most of the tested strains showed better growth at pH 4.5 than at pH 6.8, and only strain ABBC635 required alcohol or acetic acid for growth. Based on these results, we developed an acidic, Wallerstein Laboratory (WL)-based nutrient medium supplemented with 20% wine to detect wine spoilage acetic acid bacteria. The WL-based medium is easy to prepare and has high selectivity.

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### Understanding Wine Yeast Strain Variation Using a Combined Lipidomic, Metabolomic, and Transcriptomic Approach

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Under identical fermentation conditions, wine yeast (*Saccharomyces cerevisiae*) strains demonstrate variation in cell biomass formation. Yeast strains that yield



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higher biomass can complete sugar depletion more efficiently than lower biomass-yielding strains. To understand this variation in nutrient utilization efficiency (NUE), we used a multifaceted approach to assess the metabolic and regulatory differences among yeast strains. In this study, four commercial wine yeast strains with varying NUE, Montrachet, Cote des Blanc, T306, and Uvaferm 43, were fermented in synthetic MMM medium under identical anaerobic fermentation conditions. The maximum cell concentration measured by absorbance at 600 nm varied from 7.4 to 10.2 among analyzed strains. Intracellular and extracellular metabolites were analyzed using GC-MS and HPLC-RI, respectively. Complete analysis of the phospholipid profile of each strain was performed using QqQ LC-MS. Moreover, a transcriptomics approach (RNA-Seq) was taken to understand relevant transcriptional control mechanisms. Partial least squares regression of metabolomic and lipidomic data show that certain metabolic pathways, including the pentose-phosphate pathway, TCA cycle, and fatty acid synthesis, are most relevant in determining NUE. Lipid profile analysis showed that while higher concentrations of phosphotidylcholine (PC) and phosphotidylethanolamine (PE) lipids in the yeast cell membrane correlate positively with higher biomass yields, higher concentrations of phosphotidylinositol (PI) lipids have the opposite relationship with biomass yield. Finally, analyzing gene expression levels using BINGO analysis identified genes related to cellular lipids, cofactors, and amino acid metabolic processes as most related to NUE.

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## Impact of Fermentation Temperature, Yeast, and Tank Size on Dissipated Heat, Viability, and Aroma Formation in White Wine

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To ensure high quality, cooling is required during white wine fermentation. However, temperature control is the main contributor to energy demand in wineries. During fermentation, various factors influence the required heat dissipation through the cooling system. The objective of the presented work was to investigate the required heat dissipation and related energy-saving potentials for different temperature managements (14 or 19°C), yeast strains, and tank sizes (110, 1200 or 2500 L). The obtained energy savings are discussed with regard to yeast growth, release of fermentation by-products, and formation of volatile compounds. Heat dissipated by the coolant was determined with an ultrasonic flow meter coupled with probes monitoring temperature differences of the forward and return flows. High energy-saving potentials were revealed for different temperature managements and yeast strains. Depending on tank size, 65 to 90% less heat had to be dissipated to reach the same sugar level when using a temperature regime of 19°C instead of 14°C. For two different yeast strains, there were differences of 20 to 30% in dissipated heat observed in tank sizes of 1200 L and 110 L. Total yeast count and viability investigated by flow cytometry showed faster growth and faster declines in viability at higher temperature. There were also differences in the growth rate and viability between the investigated yeast strains. Analysis of yeast by-products

by enzymatic tests showed that concentrations of acetaldehyde tended to be lower at higher temperatures, while acetic acid concentrations were higher. Volatile compounds were quantified via HS-SPME-GC-MS. Differences after fermentation were more pronounced between different yeast strains than for the temperature managements. The results provide valuable experimental data on heat dissipation during fermentation and related energy-saving potentials, including their effect on essential wine quality indicators.

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### Selection of Non-*Saccharomyces* Wine Yeasts from Port Wine

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Non-*Saccharomyces* yeasts, naturally abundant during the first phase of spontaneous alcoholic fermentation, possess enormous potential to enhance organoleptic wine complexity. These yeasts are particularly relevant in the production of port wine, since fermentation is stopped prematurely through the process of must fortification. This work aimed to isolate, identify, characterize, and select non-*Saccharomyces* yeasts with biotechnological potential, from populations present in spontaneous fermentations, to be subsequently used in the vinification of port wine. To accomplish this goal, populations of non-*Saccharomyces* yeasts were isolated from different spontaneous fermentations of port wine. Species and strains were identified by PCR analysis, with selected yeast stains from the most representative strain groups subjected to phenotypic screening for relevant enological conditions associated with stress factors in must. A total of 500 non-*Saccharomyces* yeasts were isolated from eight identified species: *Hanseniaspora uvarum, Metschnikowia pulcherrima, Kluyveromyces thermotolerans, Issatchenkia orientalis, Torulaspora delbrueckii, Rhodotorula mucilaginosa, Issatchenkia occidentalis,* and *Hanseniaspora osmophila*. Interestingly, the clonal characterization evidenced a wide diversity of strains within each species. Phenotypic screening revealed that strains within the same species showed different levels of tolerance to the tested stress factors. Nonetheless, the strains that showed more promissory attributes belonged to *M. pulcherrima, K. thermotolerans, H. uvarum,* and *I. orientalis*. Sixteen of these strains were inoculated individually or in consortium in must, dominating fermentation until fortification. Remarkably, the resulting wines had equal or better organoleptic properties then wines produced with commercially available strains.

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## Enology and Viticulture – CONTINUED

### Managing pH and Acid Composition to Assess Microbial Ecology of Wine Fermentation

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This experiment was conducted to evaluate the impact of adjusting juice pH on microbial growth and metabolism. In this study, different acids and a high-acid wine were used to acidulate high pH Merlot juices grown in Washington State. Each treatment (~140 L) was carried out in triplicate for a total of 45 fermentations. The initial grape juice was 25 Brix, 4.3 g/L TA, and pH 3.8. Tartaric, D-L malic acid, and the high-acid wine (15.2 g/L TA, 5.5 g/L Malic acid, pH 2.90, and 7.4 % (v/v)) were added to control juices (pH 4.0 adjusted using $K_2CO_3$) to lower juice pH to 3.5. Additionally, the sequence of secondary fermentation was varied within each treatment (co-inoculation, sequential inoculation, and none). The acid adjustments had no significant impact on primary fermentation rate or alcohol production (14.9 ±0.2 % alcohol (v/v)). In addition, acetic acid was unaffected by the sequence of malolactic fermentation (maximum 0.4 g/L). Coinoculated fermentations finished secondary fermentation in two weeks, sequential fermentations required three to four weeks, and non-inoculated treatments finished after 50 to 70 days. Based on the data collected thus far, it appears that neither the acid adjustment nor the type of acid used has an impact on whether or not secondary fermentation occurred. Primary fermentation progress was likewise unaffected and all tanks were maintained below a maximum of $10^8$ CFU/mL. The data indicates that the timing of the inoculation was more important than the acid adjustment, with respect to microbial growth. It does appear that the rate of secondary fermentation for the coinoculated ferments was slightly accelerated by using acid additions that contained malic acid; however, the effect was trivial (~2 days). Future work will examine the impact of acid addition on the microbial populations and their metabolism. Additionally, the wines will undergo sensorial evaluation.

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### Impact of Prefermentation Cold Soak Conditions on Pinot noir Wine Volatile Aroma Compounds

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This study investigated how prefermentation cold soak conditions impact Pinot noir wine aroma. In the first year of the study, Pinot noir wines were produced from grapes that were cold-soaked for six days at 6 or 10°C, with the addition of 0, 50, or 100 mg/L $SO_2$. Six non-*Saccharomyces* yeast species, commonly isolated from grapes, were added at the start of cold soak and their populations monitored. Wine was also produced from grapes that did not undergo cold soak. At the end of cold soak, there were significant differences in a number of volatile compounds. Higher concentrations of isoamyl acetate were present in cold soaks conducted at

## Enology and Viticulture – CONTINUED

10°C than at 6°C, while higher concentrations of phenyl ethyl acetate were present in cold soaks conducted at 10°C with 100 mg/L $SO_2$. There were also significant differences in the volatile aromas of the finished wines, particularly in esters. All wines made from cold-soaked grapes had significantly higher color and polymeric pigment content than the no-cold soak wine. In the second year of the study, an addition of *Metschnikowia fructicola* was made at the beginning of a six-day cold soak. At the end of cold soak, treatments with *M. fructicola* contained significantly more higher alcohols and lower acetate esters. In the finished wines, there were significant differences in volatile aromas between wines made from grapes that did or did not undergo a cold soak. Pinot noir wines produced without cold soak had significantly higher ethyl esters such as ethyl butyrate, ethyl isobutyrate, and ethyl octonoate. Wines made with *M. fructicola* also contained significantly higher concentrations of beta-citronellol than the no-cold soak treatment.

*Funding Support: Oregon Wine Board*

### A GC/MS Method for the Elucidation of Volatile Aroma Compounds in Commercial Gins by HS-SPME

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While many styles of distilled spirits follow typical recipes due to tradition or regulation, gins are unique in the variety of flavoring adjuncts used to impart the distinct aroma attributes with which they are commonly associated. While the piney notes imparted by juniper berry additions are most widely associated with typical London dry gin character, other botanical adjuncts are responsible for characters ranging from "citrus" to "herbal" to "floral," allowing a varied assortment of styles. By examining the volatile fingerprint of any given gin, the gin's primary aroma character may be evaluated without knowledge of specific additions made by the distiller. For this purpose, an optimized method using headspace solid-phase microextraction (HS-SPME) gas chromatography/mass spectrometry (GC/MS) was developed for volatile fingerprinting of un-aged distilled spirits. In a set of 25 commercial gins, 46 common volatile aroma compounds were uniquely identified, and through use of internal standards and external calibration, 22 of these compounds were individually quantified. By correlating these fingerprints with sensory data, a clearer understanding of the roles these compounds play in gin aroma can be deduced. Future studies involving cryotrap GC-O/MS can help further elucidate the role of these compounds and how their individual and combined proportions influence gin aroma profiles.

*Funding Support: Washington State University Start-up funding*



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## Enology and Viticulture – CONTINUED

### Contribution of the Mexican Academy to Scientific Research in Viticulture and Enology in Latin America

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International competition in the wine industry and trade have increased dramatically in the last thirty years. This growth was triggered largely by wine globalization, during which new wine producing and exporting countries have emerged. In the last decade alone, wine consumption has declined in countries traditionally considered to be wine producers and consumers (e.g., Spain and France), and they compete with countries such as Argentina, Chile, and Australia. Mexico has also been part of the process of wine globalization, reflected in the 12% growth in national consumption from 2010 to 2015. However, domestic wine production has decreased, while foreign wine production has been the primary beneficiary of the growth in the Mexican market. This highly competitive environment intensifies the need of the Mexican wine sector for empirical, scientifically obtained evidence and technological innovations to guide better management choices in both the field and winery. This paper shows the results of a systematic evaluation of the contribution of Mexican academics, universities, and research centers to Latin American scientific, refereed literature in viticulture and enology. Mexican academia has failed to keep pace with the growth of the Mexican wine industry, and article production in México is significantly lower than in similar Latin American countries like Brazil, Chile, Argentina, and Uruguay; the research published is highly dependent on foreign research and is not diverse, focusing primarily on three areas: grape chemistry, agricultural sciences, and medicine.

*Funding Support: none*

### Investigating Fruitiness Aroma Perception in White Wines

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Wine aroma is complex and many different compounds can be responsible for various aromas. White wine aroma is created by compounds with direct impact, such as volatile thiols, and compounds that interact with other wine components. In this study, we investigated the aroma chemical component interactions influencing fruitiness perception of white wines. A neutral Oregon Pinot gris wine was produced and aroma was removed by adding 1g L LichrolutEN. A combination of compounds was added to the wine, forming the aroma base. These aroma base compounds are present in all wines. Treatments investigated a range of different chemical compounds such as esters, terpenes, alcohols, and thiols. Treatment aroma compounds were added to the base wine at different concentrations and combinations. Over several sensory sessions, trained panelists evaluated the different fruity aromas of the treatment wines. Panelist performance was determined using REML, and canonical variate analysis was used to relate wine chemical composition to sensory perception. Results show a relationship between terpenes and stone fruit

## Enology and Viticulture – CONTINUED

aromas and between volatile thiols and tropical fruit aromas. Other aroma compounds in combination with terpenes were found to alter the type of fruity aroma. The results of this work will help when developing wine styles and understanding of white wine quality.

*Funding Support: American Vineyard Foundation*

### Chemical and Sensory Effects of Cofermentation and Postfermentation Blending of Syrah with Selected Rhône White cvs.

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A control wine (100% Syrah) and five Syrah blends (consisting of additions by weight of 10% pressed solids of Marsanne, Roussanne, Viognier, Picpoul blanc, and Grenache blanc during crushing) were made on an experimental scale. In addition, juice from each white cv. was fermented separately and blended at a 10% rate into finished Syrah wine after malolactic fermentation to compare the effects of cofermentation and postfermentation blending. It was hypothesized that the addition of white grape solids could result in color enhancement and a different phenolic profile relative to 100% Syrah, as opposed to postfermentation blending. Wines were followed during the winemaking process to assess the effect of white fruit addition on wine chemistry, color, and phenolic composition, and up to 14 months of bottle aging. At pressing, tannin and polymeric pigment content were not significantly affected in any treatment, while catechin and anthocyanin content were. Syrah-Viognier and Syrah-Marsanne had more anthocyanin content than other cofermentation treatments, although still lower than the control. There was a statistically significant reduction in color among cofermented wines relative to 100% Syrah, with Syrah-Grenache blanc showing statistically larger reduction in color (chroma, a*) than other cofermentation treatments. Full spectrum color analysis revealed that all postfermentation blended wines had higher absorbance in the 500 to 540 nm range both after seven and 14 months bottle aging. Conversely, cofermentation generally lowered color relative to the uncofermented Syrah wine at these two time points. Although total anthocyanins, polymeric pigments, and total phenolics changed during bottle aging, no significant interaction was found between wine age and treatment, indicating that the cofermentation treatment had no effect on the evolution of phenolic profile post-fermentation. A detailed anthocyanin characterization by HPLC and a complete descriptive sensory analysis of the wines are also reported.

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## Enology and Viticulture – CONTINUED

### Understanding the Composition of White Wine Lees during Vinification

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Although racking is crucial for making good-quality wines, little is known about wine lees. In this study, we analyzed the composition of lees obtained from zero racking (juice settling), first racking (immediately after alcoholic fermentation), and second racking (before bottling) during vinification in Chardonnay white wine-making. Each of the lees was divided into two to three layers by centrifugation. Proteins, phenolics, carbohydrates, and organic acids in each layer were analyzed. The total amounts of these four recovered components ranged from 82.6 to 124%. Compositional diversity was observed among different lees layers from different racking steps. Insoluble polysaccharides from grape were the major constituents in the upper and middle lees layers from zero racking. The high proportions of proteins and mannose present in the upper and middle layers from the first racking and the upper layer from the second racking indicated that yeast cells produced during alcoholic fermentation mostly settled in those layers. Large amounts of tartrate were deposited in the lower lees layer from all racking steps, particularly the first and second racking, and this layer was mainly composed of tartrate. The content of phenolic compounds in the entire lees was low. SDS-PAGE showed that the lees contained different proteins than wine.

*Funding Support: University of Yamanashi*

### Changes in Lipid Composition of Pinot noir Wines in Response to Yeast Product Addition and Fermentation Temperature

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Firm tissues of grapes and yeast are the major sources of lipids in wine. Variation of yeasts and grape varieties could impact concentration and composition of lipids. Lipid metabolism is also affected by changes in fermentation temperature. The purpose of this study was to examine changes in lipid composition of Pinot noir wines in response to different fermentation temperature and the addition of different types and amounts of yeast derivative products. Oregon Pinot noir grapes from 2017 were fermented at 8 and 27°C. Following primary and malolactic fermentation, the yeast products Autolees and Oenolees (Laffort, USA) were added to the wines for eight weeks. Treatments included single addition of Autolees (0.3, 0.175, and 0.05 g/L), Oenolees (0.4, 0.3, and 0.2 g/L), and a mixture of Autolees (0.3 g/L) and Oenelees (0.4 g/L). Bligh and Dyer lipid extraction method with a solvent mixture of chloroform/methanol was used to extract total lipids in the experimental wines. Lipids extracted were applied onto thin-layer chromatography for purification and separation. Five lipid classes were identified as polar lipids (PL), sterols (ST), free fatty acids (FFA), triglycerides (TG), and cholesterol ester (CE). Fatty

acid profile was analyzed by gas chromatography-mass spectrometry (GC-MS). The results indicate that wine style and wine quality could be determined by lipid composition in wine. Fatty acid profile and the polyunsaturated fatty acid (PUFA) ratio n-6/n-3 could potentially be used to evaluate lipid quality in wine. It is possible that lipids may interact with other compounds in wine, such as tannins, to alter mouthfeel perception.

*Funding Support: E & J Gallo Winery*

### Chemical Profiling of Red Wines Using Surface-Enhanced Raman Spectroscopy

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The chemical profile of red wine is considered as an important criteria to assess overall red wine quality. Recently, surface-enhanced Raman spectroscopy (SERS) has emerged as a tool with good potential for wine research. Herein, a comprehensive method was developed to analyze components in red wines via a fabricated, nanoparticle-based mirror SERS substrate. A water-immiscible hybrid solvent was designed to mix with red wine samples, and two separated phases were created after dissolution. The aqueous wine phase was tested with mirror substrate and was observed sharing similar Raman spectra with condensed tannins (i.e., a major component of red wine astringency) from grape extract, where signature peaks were further characterized as NADH (i.e., a coenzyme from plant tissues). Therefore, NADH was considered as an indicator to quantify condensed tannins in red wine because both are released from grape tissues during fermentation and further statistical analysis also confirmed their correlation. In the solvent phase, NADH was eliminated due to high water solubility and interesting components (e.g., condensed tannin, resveratrol, anthocyanins, gallic acid, and $SO_2$) were extracted from red wines. The solvent phase was subsequently incorporated with silver nanoparticles to fabricate the mirror substrate and to concentrate bioactive components to SERS active domains. With the SERS analysis, five interested compounds were successfully identified in the wine extract through spectra matching analysis, which demonstrated the capability of SERS in profiling red wine chemicals. Overall, this study demonstrated a simple method to quantify condensed tannins in red wines and developed a multicomponent approach to assess the quality aspects of red wines in astringency, color, and health benefits.

*Funding Support: NEC Laboratories America, Department of Food Science, UMass Amherst*



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## Enology and Viticulture – CONTINUED

### Comparative Analysis of the Total Carbohydrate Composition of Red Wine Polysaccharides

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The chemical nature of red wine mouthfeel has been addressed in multiple recent studies. However, most focused on polyphenols such as tannins, which have been shown to account for the astringency of red wine. In contrast, the compounds contributing to mouthfeel and body remain unclear. This ongoing study investigates red wine polysaccharides, since they might affect the textural sensation, and thus the mouthfeel properties, of red wine. A procedure including precipitation, hydrolysis, and derivatization (silylation with TMSI) was developed to analyze the total carbohydrate composition by means of their per-*O*-trimethylsilylated methyl glycoside derivatives via GC-FID. Variations in the total carbohydrate composition of several red wines were observed. Although all samples comprised the same monomers (mannose, arabinose, galactose, rhamnose, galacturonic acid, glucose, and xylose), the ratios differed. Depending on the monomer, this can give insights into the winemaking process. For instance, a high-mannose portion could result from a longer yeast contact, since mannoproteins originate from yeast cell walls. However, it remains to be investigated which parameters most influence the composition (e.g., winemaking process, grape variety, or age), and to what extent this affects mouthfeel. Additional sensory studies will examine the organoleptic impact of red wine polymers to elucidate potential correlations between analytical and sensory data and to gain a better understanding of the nonvolatile sensory active compounds in red wine.

*Funding Support: Oregon Wine Research Institute*

### Advances in Quantitative Analysis of Wine Phenolics and Color Using Simultaneous Absorbance, Transmission and Fluorescence Excitation Emission Mapping Spectroscopy

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Both the color and phenolic composition of grape juice and wine are recognized as key quality characteristics associated with visual perception, taste and mouthfeel. Because the phenolic composition indicates fruit ripeness, it can be used to optimize viticulture during the veraison period, then throughout the winemaking process to establish quality control guidelines. Conventional chromatographic analyses of colored and phenolic compounds are costly, slow, and labor intensive; reagent based colorimetric assays are also slow, and importantly, do not provide compound discrimination. This study presents practical application examples of a new rapid method for discriminatory phenolic compound analysis. The method involves a patented purely optical instrument capable of simultaneous absorbance, transmission, and fluorescence excitation-emission mapping (ATEEM) spectroscopy. The ATEEM method provides both the transmission information for a com-

plete International Commission on Illumination (CIE) tristimulus analyses and the absorbance information needed for the hue, intensity, and various other parameters considered to be conventional in the wine industry. Importantly, the study demonstrates the effective and unique synergistic capacity of simultaneously analyzing the complete multi-dimensional ATEEM data set. Results are presented using unsupervised multivariate component analyses and calibrated least squares regression methods for precise sample classification and phenolic quantification, respectively. The new application examples are evaluated with respect to characterizing and classifying juice and wine samples as a function of ripening varietal and process related parameters, including possible screening for adulteration and storage issues, such as oxidation and microbial spoilage.

*Funding Support: Horiba Instruments Inc.*

## Ultrasound Application in Winemaking

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We examined the effects of cavitation induced by ultrasound on different winemaking phases, in particular, skin maceration, yeast lysis, color evolution, and lees filterability. An experimental design was created to optimize ultrasound conditions like time, amplitude, and frequency, depending on the product treated and enological objective. Tests that gave best results in the laboratory were compared to traditional enological practices. For skin extraction, best results occurred using 90% amplitude for 3 to 5 min at a frequency between 20 and 27 kHz, which significantly increased total phenolic compounds. Ultrasound of crushed grapes before vinification significantly reduced maceration time of red grapes (up to 50%) and white grape maceration for aroma extraction could be avoided. To evaluate the effect on yeast cells after fermentation, some ultrasound trials were performed on fine lees from white wines, leading to a significant rise in yeast soluble cell compounds. This increment implies a reduced aging period on lees compared with conventional techniques. There was also a significant effect of ultrasound treatment on juice and wine clarification lees that increased the tangential filtration performance in liquid recovery and treatment cost. The effect of ultrasound on tannins and anthocyanin evolution was tested on young red wines, in order to investigate the changes during the aging process. Good results were reached in each test and the use of ultrasound improved tannin polymerization and color stability. An industrial plant was made for winery application. Pilot scale application in different viticulture regions around the world confirmed the benefits of different applications using a few minutes of treatment. Technological factors (time and amplitude) must be calibrated depending on the specific enological application.

*Funding Support: TMCI-Padovan S.p.a. V.Veneto (Italy)*



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## Enology and Viticulture – CONTINUED

### Genetic Basis for High $SO_2$ Production by a *Saccharomyces cerevisiae* Wine Strain

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While USDA regulations for organic winemaking prohibit deliberate addition of sulfite to wines, they do not prohibit the presence of naturally-occurring sulfite. This contrasts with the regulations in the European Union that allow the use of sulfite as a mild antimicrobial agent and antioxidant, which disadvantages U.S. winemakers. Previous studies have found that sulfite production during fermentation by wine strains of *Saccharomyces cerevisiae* is variable and that certain strains appear to produce enough to potentially substitute for the additions prohibited by USDA regulations. Such strains could be useful to winemakers who are already making organic wine or who are interested in entering the organic market, particularly for white wines made without malolactic fermentation or barrel aging. We have found that nitrogen availability during fermentation is one source of variability and have analyzed the effect of variable levels and different forms of assimilable nitrogen on sulfite production. We have also found that establishing a genetic basis for the inheritance of "high sulfite production" among progeny of wine × laboratory strain hybrids was complicated by the observation that laboratory strains cannot ferment must under normal winemaking conditions. To avoid such artifacts related to genetic background, crosses between "high" and "low" sulfite-producing wine strain derivatives were initiated and sulfite production is being evaluated under uniform growth conditions. Genetic and genomic analyses of the progeny are on-going and are expected to reveal the genetic requirements for the "high sulfite production" phenotype.

*Funding Support: USDA-ARS Pacific NW Center for Small Fruits Research*

### Complex Complexes – Stability of Copper Sulfide Precursors of Hydrogen Sulfide under Varying Brine Dilution Conditions

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The appearance of hydrogen sulfide ($H_2S$) and related sulfur-like off-aroma (SLO) compounds in reductive storage environments presents a significant challenge to winemakers. Recent work has established that there are multiple pools of SLO precursors in wine, including soluble copper-sulfhydryl complexes. Due to their low concentrations and instability, these complexes are challenging to measure directly in wine. However, copper-sulfhydryl complexes are disrupted in the presence of strong brine, and complexes can be quantified indirectly by measuring $H_2S$ or other sulfhydryls following dilution of a sample in a concentrated NaCl solution. The concentration of copper-sulfhydryl complexes also correlates with sulfhydryl release during storage, particularly for $H_2S$. However, this correlation is imperfect, possibly because different components of the brine-releasable $H_2S$ pool differ in their stability during reductive storage. In initial work to evaluate this hypothesis,

we investigated the mechanism by which brine dilution promotes $H_2S$ release from copper-sulfide complexes. Real and model wines were prepared with different copper and sulfide concentrations, and brine-dilution assay parameters varied. Quantitation of free or released $H_2S$ was performed using commercial gas detection tubes. Preliminary results suggest a pH dependence of copper sulfide stability in model solutions treated with brine and that the efficacy of brine dilution in disrupting copper sulfhydryl complexes is inhibited at higher relative copper and sulfide concentrations.

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## Postfermentation Production of Acetaldehyde by *Saccharomyces cerevisiae*

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Postfermentation formation of acetaldehyde through practices such as microoxygenation seldom occurs predictably. Recent studies have suggested that acetaldehyde, while normally thought of as a product of chemical oxidation, may be produced by *Saccharomyces cerevisiae* in oxidative conditions. It was first hypothesized that providing oxygen after fermentation induces a switch to respiratory metabolism, during which ethanol is reverted to acetaldehyde. Dry synthetic wines made with various strains of *S. cerevisiae* to avoid loss of acetaldehyde in reactions with natural wine constituents and to evaluate the strain-dependence of acetaldehyde production, were oxygenated postfermentation. While acetaldehyde dynamics were strain-dependent, no accumulation was observed, with concentrations instead unchanging or declining for all strains tested. It was then hypothesized that acetaldehyde production is not due to respiratory metabolism, but rather to the fermentation of residual sugar stimulated by the provision of oxygen. Following the addition of 3 g/L glucose to otherwise dry synthetic wine made with *S. cerevisiae* strain EC1118, an acetaldehyde increase concomitant with the consumption of glucose was observed when the wine was oxygenated. Glucose was also consumed in non-oxygenated wine, though no acetaldehyde accumulation was observed. It is proposed that fermentation predominates regardless of oxygen availability, but oxygenation precludes the need for ethanol production normally required to maintain the redox balance of $NAD^+$/NADH, thus effectively halting the fermentative pathway at acetaldehyde. These experiments help explain the erratic acetaldehyde production observed during postfermentation oxygenation, though additional experiments with real wines are necessary to verify these findings.

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## Enology and Viticulture – CONTINUED

### Tracking Redox Potential during Fermentation as an Enological Parameter and Indicator of Yeast Metabolism

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Redox potential is an electrochemical measure of the reactive chemical environment within a solution that determines the rates and types of electron transfer reactions that can occur in a must/wine matrix. The redox potential is buffered by many reduction-oxidation couples and organic/inorganic complexes with metal species that are highly pH-dependent. Previous research has shown that the redox state of fermentation has important implications for the rate, yeast metabolism, and hydrogen sulfide ($H_2S$) formation. When the redox potential of a fermentation drops below a certain point, elemental sulfur is reduced to $H_2S$, providing a spontaneous chemical pathway for $H_2S$ formation independent of the yeast methionine pathway. Platinum electrodes can be used to measure the oxidation reduction potential (ORP), a quantifiable measurement of the tendency of a molecule or ion to gain or lose an electron. Tracking ORP provides valuable insight into the dynamics of fermentation beyond just Brix and temperature. From our observations, the ORP value of must starts high (300 to 400 ORP) but will begin dropping rapidly with the onset of yeast activity. Regular spikes are seen every 12 hrs, corresponding to pump-overs. As yeast enter into exponential growth, the ORP drops dramatically. We hypothesize that when the redox drops below ~200 to 150 ORP, the fermentation enters a reductive danger zone where the elemental sulfur can be spontaneously reduced to $H_2S$. From our data, nutrient and oxygen additions had a significant impact on both the fermentation rate and ORP values. Ultimately, it is the yeast metabolism driving the drop in ORP, with the lowest values corresponding to the yeast reaching a biomass maximum and ceasing growth. We have demonstrated that tracking redox potential as ORP is a valuable enological parameter for analyzing fermentation dynamics in real time.

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### Approaches to Limit S Off-Flavors during White Wine Fermentation with Specific Emphasis on Yeast Nitrogen Nutrition

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Low molecular weight volatile sulfur compounds are associated with reductive off-flavor in wines. Their characteristic odors range from rotten egg to rubber at very low concentrations. The formation of 15 sulfide off-flavor compounds during white wine fermentation was monitored using a novel HS-SPME GC-PFPD method and SIDA quantification. Total yeast population and yeast viability were determined by flow cytometry. Since it is known that glutathione can buffer nitrogen stress in yeast but can also lead to an increase of S off-flavors, the effect of

## Enology and Viticulture – CONTINUED

GSH addition was investigated. Fermentations with Riesling musts showing low NOPA concentrations were carried out in triplicate, whereby we first evaluated the effect of diammonium hydrogen phosphate (DAHP), pantothenic acid, or a yeast autolysates-based nutrient (IDY). It could be shown that only the addition of 0.6 g/L IDY could increase fermentation speed and complete fermentations. The addition of IDY and GSH led to a significant increase in $H_2S$ formation; the lowest off-flavor concentrations were achieved with DAHP addition, although no S-methyl thioacetate was formed. Second, we investigated the effect of must oxidation, again under different N-nutrition regimes. Total yeast cells and yeast viability increased with must oxidation, leading to faster fermentation with no significant difference in S off-flavors. The addition of IDY improved yeast viability independently of must treatment, with significant increase in $H_2S$. The addition of GSH (50 mg/L) did not affect yeast viability, but increased significantly the negative effect of IDY addition regardless of the S off-flavor investigated. In all fermentations, the use of DHAP significantly reduced S off-flavor formation and completely prevented both S-methyl thioacetate and S-ethyl thioacetate development over the whole fermentation process. Under nitrogen deficient conditions, GSH can be used in white winemaking when combined with DHAP.

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### Investigating the Effects of Temperature and Ethanol on Proanthocyanidin Adsorption to Grape Skin Cell Wall Material

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Fermentation temperature and ethanol concentration are two factors that greatly impact the extraction of phenolics from grape skins and seeds during the making of red wine. Additionally, it has been shown that grape polyphenols adsorb to the cell walls of grape skins, thereby preventing their extraction into the finished wine. Considering these phenomena, it is likely that the kinetics and extent of polyphenol adsorption to grape skin cell walls are impacted by both temperature and ethanol. Therefore, the effects of temperature and ethanol concentration on grape proanthocyanidin (PA) adsorption to grape skin cell wall material (CWM) were investigated. Adsorption experiments were conducted at various temperatures (15 and 30°C), ethanol concentrations (0 and 15% (v/v)), and starting concentration of PA (500, 1000, and 1500 mg/L). A full factorial design was implemented to investigate the impact of each variable and their interactions. PA were exposed to CWM in small, bench-top experiments, and sequential sampling was used to analyze the kinetics of the adsorption reactions. Qualitative analysis of the PA solution, using gel permeation chromatography and phloroglucinolysis, was also conducted to investigate preferential adsorption of specific molecular weights or subunit composition of PA. The extent of adsorption was shown to be dependent on both temperature and ethanol concentration. Isothermal modeling was applied for each

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temperature-ethanol condition, and equilibrium constants were calculated. Elucidating impact of polyphenol adsorption to grape-derived cell walls will ultimately increase our understanding of the limitations of phenolic extraction in the making of red wine, and thereby enhance the winemaker's ability to predict and control the chemical and sensory properties of the finished product.

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### Characterization of Vineyard and Aging Effects on Anthocyanin Profiles of Pinot noir Wines

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Anthocyanins are important flavonoid compounds that contribute to the color and overall quality of red wines. This study seeks to understand how vineyard site impacts the quantity and behavior of anthocyanins in Pinot noir wines throughout wine aging. Pinot noir wines were made from grapes of the same clonal material on 15 different vineyard sites located throughout California and Oregon. Grapes were transported to the UC Davis Teaching and Research Winery and processed, fermented, and aged under repeatable, experimental parameters. Wines were fermented in quadruplicate, blended, racked into kegs postfermentation, and bottled to age. Wines from the 2015 vintage were sampled at three points: three months postfermentation (from the stainless steel keg), eight months postfermentation (from the screwcap bottle), and 20 months postfermentation (from the screwcap bottle). These wines were characterized by quantifying anthocyanin and polymeric pigment concentrations with a high-performance liquid chromatography method with UV-vis detection. The data were analyzed using analysis of variance measuring for the effects of vineyard and age. Malvidin-3-glucoside (M-3-g) was consistently present in the highest concentration. The ratio of M-3-g to all other anthocyanins ranged from 2.5 to 6 initially, while after 20 months, the range was ~2.5 to nearly 8. Delphinidin-3-glucoside, with its tri-hydroxyl substitution, was anticipated to decrease more rapidly relative to M-3-g. The ratio of D-3-g to M-3-g was determined, however, to range from 0.033 to 0.094 at three months and from 0.030 to 0.097 at 20 months, with one exception. The anthocyanin concentration of some wines appeared to reach a pseudo steady-state between the second and third sampling point. These outcomes may be a result of our consistent winemaking and aging protocols, which minimize oxygen exposure. Future analysis will examine whether anthocyanin concentration continues at near steady-state under these bottle aging conditions.

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### Effects of Whole Cluster and Dried Stem Additions on Color, Phenolics, and Sensory Properties of Pinot noir Wines

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We analyzed the effect of whole cluster fermentation (WC) at rates of 50% (50%

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WC) and 100% (100% WC) and of dried stem additions (DS) on Pinot noir (clone 777) from the Edna Valley AVA of California's Central Coast. Wines were produced at industrial scale with treatments replicated three times in two consecutive vintages. In 2016, treatments affected most phenolic and chromatic parameters, with the exception of anthocyanins. After maceration, catechin and tannin levels were significantly higher in 100% WC and DS wines, while after nine months of bottle aging, wine color (AU 420 + 520 + 620 nm), tannins, and polymeric pigments were significantly higher, again, in 100% WC and DS wines. Additions of WC and DS generally increased pH (by 0.1 units in 2016 and by 0.18 units in 2017), and addition of 100% WC increased acetic acid in the final wines; this occurred consistently over the two vintages. In 2017, WC addition generally lowered anthocyanins but, together with DS, increased tannins and large polymeric pigments; however, there were no differences in wine color after malolactic fermentation. Sensory descriptive analysis of the 2016 wines after three months of bottle aging by a trained panel (n = 8) uncovered clear sensory effects of WC and DS additions relative to control wines. Although wines were aged in neutral barrels, only control wines displayed significantly higher oak aroma. Little sensory differences were found between 100% WC and 50% WC wines, with wines from these two treatments being perceived as more vegetal, higher in cooked aromas, and more astringent. Conversely, DS wines showed higher brown hue and enhanced berry and herbal aromas, suggesting that they were aromatically more diverse than their control and WC wine counterparts.

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WED/THUR POSTER ABSTRACTS

### Impact of Vineyard Site and Clone on Phenolic Composition of Cabernet Sauvignon Wines

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Little is understood about the influence of vineyard location on wines made from the same varietal clone, particularly regarding phenolic composition. This experiment aims to compare effects of vineyard site and clone on the chemical and phenolic composition of Cabernet Sauvignon wines in the Columbia Valley. Cabernet Sauvignon clones (FPS Clone 08 from Concannon Vineyards and FPS Clone 10 from Neustadt, Germany), both located in two mesoclimatic vineyards, were monitored throughout veraison based on a random sampling block design. Berry samples were analyzed for pH, titratable acidity, total soluble solids (Brix), yield, and phenolic composition. Wines were fermented in triplicate (12 total) in 220 L stainless steel fermenters. Pressed wine samples were analyzed for pH, titratable acidity, and phenolic classes after alcoholic and malolactic fermentation. In harvest samples, the yield, total soluble solids (Brix), titratable acidity, and phenolic composition were different across each vineyard location site (yield: $p = 0.004$, TSS: $p < 0.001$, TA: $p < 0.001$), while berry weight was significantly different by clone ($p = 0.007$). Clone had a significant effect on pH and titratable acidity (pH: $p = 0.026$, TA: $p < 0.001$), while site significantly impacted all phenolic classes

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($p < 0.001$) except polymeric pigments. Principle component analysis of the primary fermentation sample data supports the observation of a strong site influence. The initial results suggest that vineyard location is more influential on wine phenolic composition. Future work includes additional vintages and examining aspects of viticulture that impact phenolics (vine vigor, water deficits, nitrogen deficiencies). We also plan to do sensory analysis to further examine the association between vineyard site and Cabernet clone on wine quality and phenolic composition.

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

## Uncovering Winemaking × Clone Interactions in Pinot noir: Effect of Microwaved Stems and Extended Maceration

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Three Pinot noir clones (2A, 115, and 777) grown in the Edna Valley AVA were made into wine by conducting triplicate fermentations of selected winemaking techniques. In addition to an untreated control, fruit from each clone was subjected to both extended maceration for 30 days (EM) and stem addition at a 100% rate. Prior to stem addition, stems were ozonated and microwaved (10 min to 45°C) and placed at the bottom of the fermentors (MW + Stems). The clone 115 wines had 32% less anthocyanin content than 2A or 777. MW + Stems and EM decreased anthocyanins by 9% and 21%, respectively, relative to control wines. Tannin content was the most affected variable impacted by the treatments, with early effects carried over post malolactic fermentation. At day 120, MW + Stems and EM increased tannins by 732 and 772%, respectively. Wines from clone 2A were negatively impacted by EM and MW + Stems in respect to polymeric pigment formation, while clone 115 responded favorably to the MW + Stems treatment, and clone 777 responded favorably to both MW + Stems and EM. This trend was also mirrored by corresponding positive effects of MW + Stems and EM on wine color at day 120, but only for clones 115 and 777. Confirming these observations, a two-way ANOVA revealed that the only significant treatment × clone interactions were for parameters associated with polymeric pigment formation. On the other hand, absence of significant treatment × clone interactions for the remaining phenolic parameters suggests that both MW + Stems and EM affected the extraction of anthocyanins and tannins in a similar way, irrespective of the clone. Clone 2A seems to be the less responsive clone to the applied winemaking techniques from the perspective of color, phenolics, and polymeric pigment formation, although EM and particularly MW + Stem caused a dramatic increase in the tannin content of the final wines.

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## Enology and Viticulture – CONTINUED

### Adsorption Potential of Grape Insoluble Polysaccharides to Red Wine Phenolics

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Muscat Bailey A (MBA, hybrid of Muscat Hamburg × Bailey) is native to Japan and the predominant grape for making red wine in Japan. We studied the unique mechanism of phenolics extraction in MBA by comparison to that in Cabernet Sauvignon (CS). Our previous experiments revealed that anthocyanins and skin tannin were extracted during the initial period of maceration. However, their amounts decreased steeply in both CS and MBA. Seed tannin was extracted in late maceration from CS but was extracted in very small amounts from MBA. We focused on the mechanism of the steep decrease in phenolics, which might be caused by adsorption of phenolics onto insoluble polysaccharides (IPs) in grapes. Anthocyanin, skin tannin, and seed tannin were prepared and adsorption experiments were carried out using skin, pulp, and seed IPs obtained from the same grapes. In CS, skin IPs adsorbed 12% of the anthocyanins and pulp IPs, and seed IPs adsorbed 4% of anthocyanins. In MBA, skin IPs adsorbed 20% of the anthocyanins, and pulp and seed IPs adsorbed 5 and 3% of anthocyanins, respectively. In both CS and MBA, pulp and seed IPs adsorbed less anthocyanins than skin IPs. Skin and pulp IPs adsorbed 30 and 17% of skin tannin in both CS and MBA, respectively. However, seed IPs adsorbed 18% of skin tannin in CS and 45% of skin tannin in MBA. The results suggest that grape IPs, particularly skin IPs, have high affinity to skin tannin. Skin and pulp IPs adsorbed 25% of seed tannin in both CS and MBA, but seed IPs adsorbed more than 40% of seed tannin in MBA. This strong affinity of seed IPs to seed tannin may be the reason why MBA wine has so little seed tannin.

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### Effects of Variations in Berry Size and Manipulations of Fermentation Solids in Zinfandel Grapes and Wines

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Zinfandel berries were segregated into four berry size classes: raisins (9.6% of distribution), 10 mm (18.5% of the distribution), 12 mm (30.5% of the distribution), and 14 mm (41.4% of the distribution), including unsorted berries, with all treatments made into wine. Berry surface increased linearly with berry size ($R^2$ = 0.9912), but the solid to liquid ratio decreased with increasing berry size. With the exception of wine made from raisins (<9 mm), which had 168% more phenolics and 143% more polymeric pigments than unsorted berries, extraction patterns of anthocyanins, color, and tannins during fermentation were unaffected by berry size. We also manipulated berry size by adding must and fermentation solids to mimic the solid to liquid ratio of selected berry size classes. Saignée (at 29%) to mimic 12 mm in 14 mm berries only increased tannins by 22%. Addition of 36% must to 10 mm berries to emulate 12 mm berries diluted anthocyanins and



*indicates corresponding author

tannins by 68 and 65%, respectively, and addition of 29% must in 12 mm berries to simulate 14 mm berries diluted anthocyanins and tannins by 55 and 70%, respectively, indicating that the initial tannin and anthocyanin content of the 10 and 12 mm berries was the limiting factor on extraction of these phenolics. Addition of 36% solids to the 12 mm berries to emulate 10 mm berries decreased anthocyanins by 36% relative to 10 mm, and increased tannins by 48%. We conclude that berry size cannot be easily compensated in Zinfandel; even though larger berries have a comparatively lower solid to liquid ratio than smaller ones and should be amenable to compensation by saignée or by addition of extra solids, these practices seem to result only in positive effects on tannin extraction but do not affect anthocyanin extraction.

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## Increased Extraction in Pinot noir with High Concentrations of $SO_2$ at Crush

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Adding sulfur dioxide ($SO_2$) at crush is common in wine production for its antimicrobial and antioxidant activity. $SO_2$ additions also increase color extraction during maceration and fermentation. The aim of this study was to investigate the extraction of phenolic compounds and to observe alcoholic and malolactic fermentation kinetics with increasing rates of $SO_2$ added to Pinot noir at crush. Pinot noir from a single vineyard block was sorted, destemmed, and divided into four equal 0.75-ton lots. All winemaking parameters between lots were identical except for increasing rates of $SO_2$ additions made prior to fermentation. Additions were made as a 5% (w/v) potassium metabisulfite solution using rates of 50 (control), 100, 150, and 200 ppm. Following alcoholic fermentation and prior to pressing, 228 L of free run juice from each lot was racked to four identical and neutral French oak barrels and inoculated with 1g/hL malolactic bacteria. Lots had similar alcoholic fermentation kinetics but different malolactic fermentation (MLF) kinetics. The control lot completed MLF (<0.1 g/L) 10 days post-racking, with a linear increase in completion time relative to $SO_2$ concentration at crush thereafter up to 26 days. At 27 days postaddition, there was a 49% total increase in color intensity (420 + 520 + 620 nm) that was linear ($R^2$ = 0.97) with increased $SO_2$ addition. At 25 days postaddition, tannin concentration had increased by 28.5% between the control and the 200 ppm lot, as well as an increase of 30% in total anthocyanin content. Additionally, a 100% overall linear increase in the concentration of resveratrol ($R^2$ = 0.95) was observed between treatments. These results suggest that increased concentrations of sulfur added at crush could be used to increase anthocyanin and tannin concentration without preventing or significantly delaying malolactic or alcoholic fermentation.

*Funding Support: Chemeketa Community College Wine Studies Program*

## Enology and Viticulture – CONTINUED

### Mathematical Modeling of Anthocyanin Mass Transfer to Predict Extraction in Simulated Red Wine Fermentation Scenarios

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Anthocyanins are a class of chemical compounds found within red grape skins that account for the majority of color in young red wines. During fermentation and over time, these compounds engage in reactions that influence long-term wine stability and aging potential. As a result, understanding the influence of chemical and physical parameters on the extraction and subsequent evolution of anthocyanins is critical for production of red wines with desired sensory properties. The extraction rate and maximum concentration of anthocyanins in a fermenting red must can be controlled by winemakers through specific extraction operations, including mixing, prefermentation cold soaking, and manipulating process variables such as fermentation temperature. To better understand these effects, a factorial experiment was designed to investigate the impact of extraction temperature, ethanol, and solute (sugar) concentration on the extraction kinetics and mass transfer properties of anthocyanin monomers from prefermentative red grape solids. A mathematical model that allows the calculation of mass transport properties in both the liquid and solid phases was applied to the experimental extraction observations to quantify diffusion and mass transfer coefficients at varying conditions. These derived parameters were subsequently applied to simulated red wine fermentations with continuously changing liquid phase conditions. The impact of varying temperatures and mixing regimes was also investigated using simulations to further explore their effect on extraction rate and maximum potential to extract anthocyanins during fermentation. Results from this study could inform winemakers of the optimal process conditions to achieve a desired anthocyanin concentration and may lead to development of new process control systems for winemaking. Application of these simulations could also improve overall efficiency and decrease production costs of a winery by optimizing available tank space and minimizing energy consumption from unnecessary operations designed to extract color from red grapes.

*Funding Support: Wine Australia School of Agriculture, Food and Wine, The University of Adelaide*

### Aroma and Chemical Characterization of White Wines Produced in Mexico

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Wine aroma is formed by hundreds of volatile and semivolatile compounds. The typical wine flavor is mainly due to volatile molecules coming from grapes (*Vitis vinifera*). Chardonnay, Sauvignon, and Chenin blanc are grown in three different wine regions in Mexico, and published studies suggest that Mexican white wines made from these grape varieties contain interesting flavor notes. We explored the influence of three different wine regions on chemical and aroma quality of Chardonnay, Sauvignon, and Chenin blanc wines. Total and volatile acidity, alcoholic



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## Enology and Viticulture – CONTINUED

strength, pH, color, phenolic content, and total and free $SO_2$ of Mexican wines were analyzed according to the conventional methods. The volatile composition was determined by solid phase extraction (SPE) and gas chromatography–mass spectrometry (GC-MS) to isolate and analyze free volatile compounds. For winemaking, grapes were divided into individual batches. Each batch was treated in the standard way with minimal skin contact following the traditional method. The three grape varieties were destemmed and crushed, the pomade was mixed with 100 mg/kg $SO_2$, kept at 18°C for 24 hrs, and then pressed. Fermentations were performed in vats of 50 and 100 L. Musts were inoculated with *Saccharomyces cerevisiae* and fermentations were conducted at 18°C for two weeks. Each fermentation was carried out in duplicate. GC-MS analysis of wines identified ~30 to 60 free aroma compounds in Chardonnay, Sauvignon, and Chenin blanc wines produced in Mexico. $C_6$, terpenic and bencenic compounds were the major components of varietal aroma. The bound aroma fraction was characterized by a larger concentration of bencenic and $C_{13}$-norisoprenoid compounds, which suggest that these wines possess a great aroma potential. Each wine showed a complex chemical profile with a wealth of aromas in its global composition.

*Funding Support: Consejo Nacional de Ciencia y Tecnología (Mexico) (CONACYT)*

WED/THUR POSTER ABSTRACTS

### Sensory Comparison of Two Consecutive Years of Pinot noir Wines from 12 Vineyard Locations

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The reproducibility of sensory differences among Pinot noir wines derived from a single clone and grown on different vineyard locations along the US West Coast, was investigated over several years. We anticipated finding sensory differences among the wines from different sites and that these dissimilarities would correlate with phenolic constituents of these wines. The intention of this initial sensory experiment was to observe whether aging affected the wine within one year by verifying sensory characteristics from a single vintage in two consecutive years. The first vintage from 2015 was investigated in spring 2016 and 2017 with descriptive analysis (DA) and temporal dominance of sensation (TDS). No previous study has reported on the effect of vineyard location on temporal taste and mouthfeel characteristics. Phenolic compounds representing important fractions, including anthocyanins, hydroxycinnamates, and monomeric flavan-3-ols, were also measured and correlated with taste and mouthfeel attributes using multiple factor analysis. A two-way ANOVA of shared taste and mouthfeel attributes from the two years (sweet, sour, salty, bitter, astringent, viscous, puckering, and hot) showed no significance for the year and wine interaction. We infer therefore that our Pinot noir wines aged in a similar manner on these sensory properties. We conclude that future sensory analyses on these Pinot noir wines produced and stored under identical conditions can be performed either after the bottling date or a year later. The unique controlled experimental design, in combination with the sensory analytical tools (DA, TDS) and the phenolics composition, help clarify the impact of grapegrowing conditions on wine sensory characteristics.

*Funding Support: Jackson Family Wines*

**Bold type indicates presenting author**

## Enology and Viticulture – CONTINUED

### Shedding Light on Port Wine Aroma Production Complexity: Terroir versus Yeast Impacts

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Port wine is a fortified wine produced in the Douro Appellation (Portugal) under very specific conditions resulting from natural and human factors. Its intrinsic aroma characteristics are modulated by a network of factors: terroir particularities, grape varieties and winemaking procedures, particularly the yeast strains. Over the past three decades, targeted consistency in winemaking has led to the almost ubiquitous application of commercial *Saccharomyces* strains. Although the recent introduction of commercial non-*Saccharomyces* strains has resulted in improved complexity, the potential impact and diversity of native Douro yeast strains responsible for Port production have yet to be studied; hence, the present investigation of their impact on grapes from different terroirs. An in-depth study was conducted on the impact of the binomial "yeast strain versus terroir" on potential aroma characteristics of Port wine produced from the Touriga Nacional variety. The strategy included the analysis of wine volatile composition, sensory properties, and yeast population profiling through fermentation, permitting a comprehensive understanding of the impact of "terroir versus yeasts." The wines were analyzed using an advanced multidimensional gas chromatography methodology (HS-SPME/GC × GC-ToFMS) in tandem with ANOVA-simultaneous component analysis and hierarchical clustering analysis. Attention was principally focused on volatiles reported as exhibiting high level odor activity values in Port wines[1]. Several volatile components were determined distributed over the chemical families of acids, alcohols, aldehydes, terpenic compounds, esters, norisoprenoids, and volatile phenols. This research reveals that native strains were detected under all conditions analyzed, including those inoculated with commercial strains. Despite the significant contribution made by yeast strains, terroir had the greatest effect on Port wine aroma.

[1]Rogerson FSS and De Freitas VAP. 2002. Fortification spirit, a contributor to the aroma complexity of Port. J Food Sci 67:1564-1569.

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### Toward the Development of a Sustainable Wine Scoring System. A Case for Craft Wineries

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Sustainable winegrowing and winemaking practices are being adopted by the wine community as a key driver to adapt to climate change and natural resource depletion while fulfilling consumer expectations. Quantifying and predicting the environmental, social, and economic impact of implementing sustainable practices or programs is challenging due to the lack of multivariable metrics and normalization criteria. The main objective of this research is to develop a methodology to unify sustainability assessment for the wine industry. Our aim is to define the flow

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of energy, water, and material during winemaking under different conditions and their environmental impact, following a Life Cycle Assessment (LCA) method. As a first step, we quantified environmental impacts in distinct stages of the life cycle of craft wine in an emerging, non-traditional wine region of the United States. The LCA was performed following ISO 14040 guidelines, and the impact assessment was conducted using SimaPro 8.3, in accordance with the TRACI 2.1 /US 2008 methodology. The result is presented in 11 midpoint impact categories. For most of the impact categories, the highest process contributing to environmental impact is fertilizer use during grape production, the production of the glass bottle, and transportation during different production stages. Under the application of different normalization criteria, when explaining the environmental impact of wine, impacts such as ecotoxicity, eutrophication, and water depletion become more relevant to the carbon footprint. To define a sustainable wine scoring system (SWSS) to benchmark region, winery, and wine sustainable performance, a single-score impact assessment as carbon footprint does not adequately represent the environmental impact. The different impacts obtained by LCA must be normalized and weighed, considering the regional ecosystem boundaries.

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## Assessment of Red Wine Astringency Perception by Physicochemical and Sensory Methods

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The perception of astringency has been characterized extensively through chemistry (complexes between salivary proteins and tannins) and by sensory analysis with trained panels to predict human perception. The physical aspect/human oral physiology of wine astringency perception has not been studied until now, although saliva has been shown to be involved in mouthfeel perception. In this study, condensed tannins were extracted from two red wines (cv. Cabernet Sauvignon and Pinot noir) and added back to them and to a model wine at 0.5 g/L, prior to chemical characterization of their composition by HPLC-DAD after acid-catalysis and by sensory analysis. The chemical consequences of interactions between mucin, poly-L-proline, saliva and red wine tannins were evaluated by turbidimetry and protein precipitation assay. The friction/lubrication properties of lubricant (mucin or saliva) with red wines were measured with a surface force apparatus, including development of a method to mimic mouth lubrication. The mean degree of polymerization of Cabernet Sauvignon condensed tannin, and the haze/aggregates formation with mucin and poly-L-proline were higher than in Pinot noir tannin. The turbidity of saliva and poly-L-proline with tannins added to Cabernet Sauvignon was higher than that of tannins added to Pinot noir wine. The coefficient of friction measured for red wine, with saliva as the lubricant, was higher in Pinot noir than in Cabernet Sauvignon wine. The lower friction of Cabernet Sauvignon is likely due to exclusion of the aggregates and depletion of more polymeric and

protein material from the contacting region. The perceived dryness of red wines correlated positively with turbidity measurements with saliva, while the correlation with friction coefficient was much lower, meaning that physical, chemical, and sensorial methods must be used together to get an overview of red wine astringency perception.

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## Reverse Osmosis as a Method for Mitigating Smoke Taint

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Smoke exposure to grapes prior to vinification can lead to the presence of smoke-related volatile phenols and associated glycosides. These compounds provide negative sensory attributes to the wines, including ashy, medicinal, and smoky aromas and aftertastes. A method for alleviating this taint prior to bottling is needed to mitigate the negative impacts of smoke exposure in finished wines. Smoke-affected wines from the ongoing smoke taint project in the Collins lab, as well as affected wines from commercial wineries, were treated using a small-scale reverse osmosis (RO) filtration system, in which the smoke taint compounds migrated into a permeate stream. The permeate stream was then passed through carbon filters to remove the smoke-taint compounds. The compositions of the permeate and retentate streams were analyzed using gas chromatography-mass spectrometry (GC-MS) and ultra-high-pressure liquid chromatography-quadrupole time of flight-mass spectrometry (UHPLC-QTOF-MS). Principal component analysis of compositional data collected during an RO time study found separation between samples from the permeate and retentate streams. The permeate stream contained smaller compounds associated with smoke taint such as pyrocatechol, syringic acid, and caffeic acid, while the retentate stream contained larger compounds, including several flavonoids. Samples taken before and after the carbon filters became more similar as processing time increased, with peak areas decreasing for prefiltration samples and remaining steady for postfiltration samples for several smoke-related compounds. The treated wines are being monitored using GC-MS and UHPLC-QTOF-MS for posttreatment release of free smoke taint compounds through hydrolysis of smoke taint-related glycosides. This study serves as an initial evaluation of the kinetics of hydrolysis post-RO to determine whether RO treatment can be an effective tool for mitigation of smoke taint in affected wines.

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



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## Enology and Viticulture – CONTINUED

### Assessing Smoke Taint Risk Based on the Composition of Smoke-Exposed Grape Berries and the Resulting Wines

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Grapes from vineyards exposed to wildfires can result in wines that have aromas described as ashy, cigar butt, or smoky. In recent years, there have been increased incidences of wildfires in close proximity to viticultural areas. This study exposed trial vineyard blocks to simulated wildfire smoke for periods of 26 to 48 hours, replicating conditions seen in recent wildfire episodes in Washington and California. Research was carried out by smoking 60 vine sections of the WSU Roza research vineyard in Prosser, WA. Modular hoop houses were used to contain the smoke in the exposed block of vines and to cover a control block, which was not smoked. Chardonnay and Merlot were smoked using a mixture of steppe land vegetation, while Cabernet Sauvignon was smoked using a mulch of coniferous bark and wood. Smoke intensity was monitored during each trial via TSI DustTrak DRX 8533 particle monitors (Shoreview, MN). Wines were made from the fruit using research winemaking protocols. Samples were analyzed for smoke-related compounds and their glycosides using gas chromatography/mass spectrometry (GC/MS) and ultra high pressure liquid chromatography/quantitative time-of-flight mass spectrometry (UHPLC/QTOF-MS). Principal component analysis of the UHPLC/QTOF-MS data for finished wine samples showed separation between Chardonnay, Merlot, and Cabernet Sauvignon wines, and between smoked and control samples within each variety. Wines from smoke-exposed fruit contained guaiacol, syringic acid, dihydroxy-benzaldehyde, and glycosides of at least two volatile phenols. Preparation and analysis of the samples collected during this study and identification of additional smoke-related compounds is ongoing.

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

### Smoke Taint: Challenging Current Beliefs and Exploring In-Winery Mitigation Strategies

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It is well established that the concentration of volatile phenols and their glycosides correlate with a wine defect known as smoke taint, which can occur when fermentation is performed using *Vitis vinifera* berries that were exposed to forest fire smoke. There are conflicting reports regarding the stability of the volatile phenolic glycosides that survive fermentation, with some studies suggesting their hydrolysis can increase the intensity of smoke taint during bottle aging. Conclusive data, based on chemical stability tests and controlled small-batch fermentations, demonstrated a lack of glycoside hydrolysis in wine that will be discussed, as well as what these data tell us about other compounds that may correlate to smoke taint. *V. vinifera* naturally produces volatile phenols like guaiacol and syringol via the phenylpropanoid and/or shikimic acid metabolic pathways. An influx of exogenous volatile phenols from

smoke could change the relative concentrations of secondary metabolites associated with these biosynthetic pathways. Additionally, since phenylpropanoids are, in part, regulated by plant stress, it is conceivable that smoke exposure could induce changes in the endogenous expression of phenylpropanoids, independent of the presence of smoke-borne volatile phenols. To investigate these hypotheses, the concentrations of key metabolites in the phenylpropanoid/shikimic acid pathways were analyzed in smoke-exposed and control berry and wine samples. The impact of these studies on the objective assessment of smoke taint in grapes will be discussed. Finally, the enzymatic activity of four *Saccharomyces* strains was explored by performing fermentations on smoke-exposed berries and monitoring the volatile phenols produced following primary fermentation. The results of these fermentations will be presented as a starting point to develop in-winery solutions to mitigate the ongoing impact of forest fires.

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## Compositional Differences in the Combustion Products of High Desert Botanicals in Grapes and Wine

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The wildfires affecting the Pacific Coast states during the 2017 harvest season have increased concern over the impact of smoke on the quality of grapes and wine. With this heightened concern comes the need to evaluate the compositional differences in smoke generated by fires from different fuel sources to determine how fuel source might affect aroma and flavor in wines. This study quantitatively evaluated the compositional differences in the combustion products of 16 assorted plants native to southeastern Washington. These include sagebrush, cheat grass, rabbitbrush, and puncture vine, among others. An amount of desert plant material sufficient to burn consistently for 5 min in triplicate was collected in July 2017 and dried two to five days (depending on plant type) before being burned in a modified commercial smoker. Smoke particle concentration was monitored using a TSI Sidepak AM510 aerosol monitor (Shoreview, MN). Smoke particles were collected using Whatman glass microfiber filters (1.0 µm pore size) positioned in-line with the smoker exhaust. Three 1cm discs were removed from each filter and analyzed using solid-phase microextraction (SPME) coupled with gas chromatography/mass spectrometry (GC/MS). Preliminary results showed differences in the composition of smoke produced by burning the 16 different botanicals, with p-cresol, m-cresol, 4-ethylphenol, and syringol present in the highest concentrations in sagebrush, while the conifer bark mulch exhibited the highest levels of guaiacol and creosol. By contrast, rabbitbrush contained several late-eluting, tar-like polyaromatic hydrocarbons that were not present in most samples, but it had lower concentrations of guaiacol. Future work will focus on understanding the relationship between wildfire fuel source and the presence of various smoke taint compounds found in grapes and wine.

*Funding Support: Washington State Wine Commission*



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## Enology and Viticulture – CONTINUED

### Identification of Aroma Differences in Norton and Cabernet Sauvignon Grapes and Wines Using a Non-Targeted Analysis

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Grape-derived volatiles play an important role in wine aroma and contribute to overall wine quality. Free volatiles and nonvolatile precursors, including glycosides, are present in grapes. The aroma precursors released during winemaking due to acidic or enzymatic hydrolysis give varietal characteristics to a wine. We investigated grape-derived volatiles in Cabernet Sauvignon, a popular *Vitis vinifera* grape, and an interspecific hybrid, Norton. Although Norton possesses important viticultural traits such as cold hardiness and disease tolerance that have made it economically important to Missouri, wines made from it are less popular than the *vinifera* wines globally. While earlier efforts have been made to determine the volatile profile of hybrid wines using a targeted approach, we opted for a more inclusive, non-targeted metabolomics approach to investigate the differences in Norton and Cabernet Sauvignon grapes and wines. Both free and bound volatiles were profiled in grapes and free volatiles, in wines. Twenty-one samples of Norton and Cabernet Sauvignon grapes, from different vintages and sites, along with their 10 different commercial wines, were analyzed using headspace SPME-GCMS. Data was processed using XCMS to identify features different between the two cultivars. 825, 697, and 403 features were found to be different for free grape volatiles, bound volatiles, and wine volatiles, respectively, at a minimal 0.05 significance level and 1.5-fold change. Those features were used to identify and quantify odor-active compounds that varied in concentration, including β-linalool, β-damascenone, β-ionone, eugenol, and methyl salicylate. We found no compounds present in one that was absent in the other cultivar; however, the concentrations of the compounds identified were always higher in Norton than Cabernet Sauvignon. Identification of these differences is critical to optimize management of Norton and useful in varietal development, where the end goal is disease-tolerant fruit with a widely accepted aroma profile.

*Funding Support: USDA*

### Applications of the CRISPR Technology for the Wine Industry

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The development of targeted genome editing is expected to become a major biotechnology tool to improve winegrape production, with potential for the creation of new valuable products for the wine industry. Among the editing methods currently available, the clustered regularly interspaced short palindromic repeats (CRISPR)-associated Cas9/sgRNA system has become a preferred editing tool for crops. In our lab, we plan to develop several research programs using the CRISPR technology in conjunction with the microvine system to address several scientific questions with potential application for the industry. The microvine is a more tractable system for genetic engineering and can accelerate functional gene characterization to within a year, even at the fruit level. Using CRISPR, we propose first to target genes that

function as negative regulators of useful traits, ideal candidates for CRISPR-mediated gene editing. These traits could include, but are not limited to, mildew locus O (MLO), powdery mildew susceptible gene, viral-resistance genes, and negative regulators of nutrient uptake and/or assimilation. At the fruit level, the CRISPR CAs9 system can also be used to mitigate or to repress negative features for optimum fruit composition such as production of off-flavors produced either by the fruits or as by-products of the fermentation process. One primary target, but not limited to, could be alteration of VitviOMT3, a protein responsible for producing methoxypyrazine in Sauvignon cultivars. We will also propose to use CRISPR technology to understand long-distance communication between scion and rootstocks using a *trans*-grafting approach (genetically engineered rootstocks). Ultimately, we will explore repurposing the Cas9 genome editing system to generate grapevine mutant lines with gain/loss of gene function (CRISPRi/CRPIRa). This approach will accelerate characterization of the relationship between gene(s) and traits of interest.

*Funding Support: None*

## Determining the Role of Auxin-Response Factor 4 (VitviARF4) in the Ripening Initiation of *Vitis vinifera* Fruits

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In the light of evolving climactic conditions, control of ripening initiation will be a major trait of interest for winegrape production. The current research project aims to validate the role of VitviARF4 on the timing of ripening initiation. Three objectives were designed to achieve this goal: 1) characterization of VitviARF4 through genetic engineering using the microvine model, by either inducing or silencing VitviARF4 expression, and validation of its protein interactors during ripening; 2) identification of ripening-related genes directly under the control of VitviARF4; and finally 3) altering VitviARF4 activity to determine its effect on fruit composition at harvest. Since June 2016, we have been able to achieve several milestones on the current project. As part of objective 1, we established the microvine system at OSU and recently conducted our first attempt at genetic engineering. Using a protein-protein interaction assay, we have identified 170 potential protein partners to VitviARF4, some associated with ABA-, sugar- and ethylene-signaling. This might confirm the likely role of VitviARF4 in the interplay that takes place during the ripening initiation between hormones and sugar. For objective 2, we finalized a cloning procedure for the different constructs designed to induce or silence *VviARF4* in the microvine plants. For objective 3, we adapted a new analytical method to measure metabolites associated with organic, amino, and phenolic acids, different types of carbohydrates, polyols, and three classes of flavonoids (anthocyanins, flavonols, and monomer and dimer of tannins). For those metabolites, we built an in-house library of 95 analytes and tested it against mass spectral data from berry extracts. We identified 30 analytes covering major compounds existing in ripe grape berry. These include tartrate, malate, glucose, fructose, sucrose, and several polyphenol-related compounds.

*Funding Support: Oregon Wine Board, Erath Family Foundation and Fermentation Initiative*



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## Enology and Viticulture – CONTINUED

### Evaluation of Postharvest Marketability of Arkansas Table Grapes Grown in High Tunnel Production

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Table grape (*Vitis* sp.) production in the southeastern United States is limited by pests, disease, and environmental challenges, but could be improved by production in high tunnels. The postharvest marketability of three University of Arkansas table grape cultivars (Faith, Gratitude, and Jupiter) were evaluated. The grapes were established in 2014 on a Geneva Double Curtain trellis in a high tunnel vineyard in Fayetteville, AR. The fruit was handharvested in July and August 2017, then randomized. Two clusters per genotype were placed into a 0.9 kg clamshell in triplicate for analysis. Yield parameters at harvest showed Faith, Gratitude, and Jupiter had cluster weights of 234.84, 407.28, and 110.52 g, respectively, and yield/vine of 35.54, 35.18, and 21.93 kg/vine, respectively. Composition (soluble solids, pH, and titratable acidity) and berry firmness were evaluated at harvest, and marketability attributes (berry firmness, weight loss, decay, and berry drop) were evaluated during storage for 0, 7, 14, and 21 days at 2°C. At harvest, the grapes had soluble solids of 10.70 to 17.40%, pH of 2.91 to 3.85, titratable acidity of 0.38 to 0.76%, and firmness of 2.28 to 3.94 N. There were no significant cultivar × storage interactions for composition, firmness, decay, or berry drop, and storage did not affect these attributes. Firmness and berry drop were not affected by cultivar, but composition and decay were cultivar-dependent. Faith had the most decay (3.36%), followed by Gratitude (2.72%), and Jupiter (0.10%). There was a significant cultivar × storage interaction for weight loss, where weight loss increased during storage for each cultivar. After 21 days storage, Faith had the most weight loss (3.78%), followed by Gratitude (3.05%) and Jupiter (2.36%), but it was relatively low for all cultivars. Table grape production in Arkansas has risks, but this study showed that high tunnel table grapes had marketable fruit at harvest and during postharvest storage.

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### The Effects of Reducing Herbicide Use on Vine Performance, Productivity, and Fruit Composition in New Zealand Vineyards

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Due to increasing market pressure and because of the development of herbicide resistance in weed species, it is important for the industry to reduce, and eventually eliminate, herbicide use in vineyards. This trial was set up to assess the effects on vine growth, yield, and fruit composition of multiple herbicide sprays (the industry standard) versus a mixed management strategy of single herbicide spray before budburst, with any additional weed-control passes being nonchemical (undervine mowing or cultivation). The vineyards were set up as split plot designs with five sampling loci in each plot. The trial was conducted in three Merlot vineyards in Hawke's Bay and three Sauvignon blanc vineyards in Marlborough, New Zealand.

## Enology and Viticulture – CONTINUED

Undervine vegetation was significantly different among treatments in all vineyards from flowering onward, with mixed management having more grass and clover undervine, and multiple herbicide having more bare area. There were rarely significant differences in stem water potential throughout the season and in canopy gap percentage at flowering, veraison, or harvest, suggesting that the vegetation that grew after the first herbicide spray in the mixed treatment did not compete with the vines to such an extent that their growth was greatly limited. The reduced herbicide use did not greatly affect yield or fruit composition, demonstrating that mixing in nonchemical weed control methods is a viable option in vineyards, and one that will not engender resistance in weed species. It is hoped that the findings of this study will embolden more growers to reduce their reliance on chemical methods for weed control in vineyards and to begin to employ more nonchemical means moving forward.

*Funding Support: New Zealand Winegrowers and New Zealand Ministry of Business, Innovation, and Employment*

### Secondary Bud Growth and Fruitfulness of *Vitis vinifera* Grenache Grown on the Texas High Plains: A Two-Year Review

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In 2017, the grape and wine industry had an overall economic impact of $13.1 billion within the state of Texas. However, most Texas vineyards are subject to late spring frosts, which potentially reduce crop yields and fruit quality. If Texas grapegrowers were knowledgeable regarding cultivar secondary bud fruitfulness, secondary bud fruitfulness might influence variety selection during vineyard planning. Therefore, objectives of this experiment were to analyze data from a two-year study comparing growth and fruitfulness of shoots grown from primary and secondary buds of *Vitis vinifera* Grenache grafted to three rootstocks (110R, 1103P, and Freedom) on the Texas High Plains. Grenache vines (vertical shoot-positioned trellis, bi-lateral cordons, four spurs/cordon, and two buds/spur) were planted in a randomized complete block design in 2006. Treatments were repeated on the same vines over consecutive growing seasons (2016 to 2017) and included: primary bud remained, and following budbreak allowing shoot growth to reach 15 cm in length, then removing primary buds. Pruning weight, gas exchange, and fruiting data were collected each year. During the first growing season, gas exchange data from leaves grown on secondary bud shoots tended to have greater gas exchange rates. Second year gas exchange data indicate no differences. Pruning weights suggest greater vegetative growth of shoots grown from primary buds or on rootstock 1103P. In general, each year fruit grown on primary bud shoots had greater berry weight, cluster weight, and yield than fruit grown on secondary bud shoots (across rootstocks, yield was reduced nearly 98% in year one, and 33% in year two). Berry brix levels were also influence by rootstock and bud removal. Results of this study offer new insight into the response of Grenache vines to potential early frost damage.

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## Enology and Viticulture – CONTINUED

### Performance of Taxonomically Diverse Arbuscular Mycorrhizal Fungi Isolated from a Red-Hill Soil with Pinot noir

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Grapevines rely on arbuscular mycorrhizal fungi (AMF) to obtain ample phosphorus (P) from soils with moderate to low P, like the red-hill soils in western Oregon. Prior research using DNA sequencing indicated that six to 11 species of AMF colonized the roots of Pinot noir within a given vineyard. However, little is known about how different species of AMF function in vineyard ecosystems. We investigated the efficacy of five native AMF species representing five genera to promote growth and nutrient uptake of Pinot noir in a red-hill soil under well-watered and drought conditions. Rooted cuttings were grown in the presence of five different AMF or without AMF, and with or without moderate drought stress. After eight and 16 weeks, whole vines were destructively harvested and biomass and nutrient uptake were determined. Results showed that four of the five AMF colonized roots well, increased root and shoot biomass, and predominantly increased P uptake. However, a *Claroideoglomus* isolate was superior in promoting shoot growth and P movement to shoots. The ability to enhance vine growth and P uptake was not related to the extent of arbuscules in roots, suggesting that some P exchange may occur via hyphae. Water limitation reduced P uptake in this soil as a main effect across all AMF treatments, indicating that water stress reduced the capacity for P uptake by all AMF species studied here in a similar fashion.

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### Vine Vigor Influences Pinot noir Bud Fruitfulness to a Greater Extent than Pruning and N Practices

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Pruning and nitrogen fertilization are management practices that influence vineyard productivity. Cane pruning is predominant in Oregon Pinot noir vineyards, as producers fear that spur pruning will result in low yields due to a lack of basal bud fruitfulness. Nitrogen (N) fertilization is often avoided, because it is thought to create excess vegetative growth and reduce wine quality, but N-deficient vineyards often have reduced yields. Little information is available on the impacts of pruning and N fertilization on bud fruitfulness, an important predictor of yield. Two separate experiments were conducted in commercial Pinot noir vineyards in Oregon to understand the impacts of pruning method (cane and spur) and N fertilization (N fertilization and no fertilization) on fruitfulness and yield. It was hypothesized that basal nodes would be fruitful but might reduce yields in spur- compared to cane-pruned vines. It was also hypothesized that vines with low N status would have lower fruitfulness and yields. Year 1 pruning trial results indicate that basal buds have floral primordia, and similar bud fruitfulness and inflorescence primordium size were found in buds with both pruning methods. However, fruitfulness in

spring was higher in cane-pruned vines. Vine growth, yield, and fruit composition did not differ between cane and spur pruning. Year 1 results of the N fertilization trial indicate that N fertilization had little impact on bud fruitfulness, fruitfulness in spring, vine growth, or yield. However, vine vigor, measured in dormant canes, was related to bud fruitfulness parameters in both experiments. In the N trial, greater bud fruitfulness was related to larger cane size (weight and internode diameter). In both trials, greater inflorescence primordia size was observed with larger internode diameter. This work will continue into 2019, and the results will help improve pruning and N management guidelines for Oregon Pinot noir producers.

*Funding Support: American Vineyard Foundation*

## Early Source-Sink Modulation in Merlot (*Vitis vinifera* L.) Enhances Fruit Quality through a Flavonoid Metabolome Shift

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Removal of basal leaves early in grapevine vegetative development limits assimilation of carbohydrates to florets, reducing fertilization and the number of berries per cluster. This subsequently controls vine yield, improving fruit quality. Mechanization of this practice can save time and money for growers, but has not yet been compared with manual application in cool climate growing regions, where adequate seasonal temperature accumulation is a major limitation on yield and fruit technological maturity at harvest. The goal of this study was to compare mechanization of leaf removal with manual removal of six leaves at the prebloom (E-L 17) or after-bloom (E-L 27) phenological stages against a control (removal at veraison) over two seasons. Results indicate that mechanical treatments removed less leaf area than manual ones at each timing, leading to lower lateral compensation and poorer fruit-zone microclimate conditions. Despite this, fruit set decreased more in mechanical treatments at each timing. Photosynthesis data showed a strong compensation in Phi2 (quantum yield of Photosystem II) in apical leaves of vines subjected to prebloom treatments. Additionally, the NPQt (non-photochemical quenching) parameter was significantly pronounced in the medial and apical leaves of both manual treatments, indicating severe stress conditions in the leaves unrelated to weather conditions. Compared to manual treatments, berry sugar concentration was higher due to mechanization, while total acidity decreased only with manual treatments. Furthermore, metabolomics analysis revealed a significant increase in anthocyanin and flavonol compounds with prebloom mechanical treatment in both experimental years (2016 and 2017). Enhanced fruit quality with the mechanical treatments proved to be the result of a fruit-zone that received adequate light and temperature exposure early in development but that retained significantly more leaf area to influence ripening after veraison. This information provides an important strategy to ripen red *vinifera* cultivars in cool climates.

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## Enology and Viticulture – CONTINUED

### Effect of Shoot Density Manipulation on Canopy Growth and Berry Chemistry

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Cordon-trained, spur-pruned grapevines frequently display variation in development as a function of position along the cordon. Discrepancies in shoot growth and/or cluster ripeness can impact the timing and success of vineyard management activities such as leaf and cluster thinning and eventual fruit maturity indices at harvest. The objective of this research is to determine the role that shoots/meter manipulation has on berry chemistry and on the homogenization of shoot development down the length of a cordon. The study was conducted in Oakville, CA, on bilateral cordon-trained, spur-pruned Cabernet Sauvignon vines on 1.8 × 2.5 meter spacing. Vines were pruned to either 5.5 shoots/meter or 11.1 shoots/meter for a total of 12 or 24 buds per vine, respectively. Parameters measured included shoot length, internode length, cane diameter, and pruning and cluster weights. Berry sampling was performed as a function of position along the cordon (head, middle, and end) and encompassed soluble solids, pH, titratable acidity (TA), and total phenolics. In the first and second year of the study, implementation of 5.5 shoots/meter resulted in uniform shoot internode length, diameter, and berry chemistry down the length of the cordon. The 11.1 shoots/meter treatment demonstrated variable development as a function of position with respect to both shoots and clusters. In year three, the 5.5 shoots/meter treatment began showing trends similar to that of the 11.1 shoots/meter with respect to berry chemistry, in that nonuniformity was an issue as a function of position along the cordon. Specifically, cluster soluble solid accumulation in the 5.5 shoots/meter treatment was higher at positions originating from the head of the vine than at the end. The 5.5 shoots/meter treatment also exhibited higher berry pH at positions originating from the head and lower pH at the end cordon positions.

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### Use of Foliar Spray of $CaCO_3$ to Reduce Heat Stress and Enhance Fruit Quality of Syrah in the San Joaquin Valley of California

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Direct sunlight and heat waves in arid, warm viticultural regions can reduce fruit quality by inhibiting anthocyanin biosynthesis, promoting anthocyanin degradation, hastening acid metabolism, and causing direct yield loss due to sunburn. Such damage can cause significant economic loss for winegrape growers in the San Joaquin Valley (SJV) of California. The potential for $CaCO_3$ foliar spray to prevent such damage was investigated in 2017. A random complete block design was employed with three treatments: two rates of $CaCO_3$ plus an untreated control,

each replicated five times. Foliar spray of $CaCO_3$ using an air blast sprayer was applied monthly after fruit set, with a total of four sprays during the season. Mid-day leaf water potential (LWP) and canopy temperature were measured weekly from the onset of bloom. Fruit-zone photosynthetically active radiation (PAR), leaf gas exchange, and berry ripening were tracked weekly from the beginning of veraison. Yield components and berry composition were measured at harvest. At the dormant period, pruning weight was collected to calculate Ravaz index (kg/kg). Foliar spray of $CaCO_3$ lowered canopy temperature with little effect on LWP. Slightly higher berry total soluble solids accumulation was observed when $CaCO_3$ was sprayed. No other differences in yield or berry composition were found among treatments. When correlation analysis was performed to identify the key factors impacting berry anthocyanins and phenolics, fruit-zone PAR and pruning weight were the most significant variables. The results indicate that in the SJV, fruit zone exposure and vine vigor had a greater impact on berry anthocyanins and phenolics than $CaCO_3$ treatment.

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## Grapevine Fanleaf Virus Resistance Screening in a 101–14 x Rotundifolia Population

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Grapevine fanleaf virus (GFLV) causes fanleaf degeneration, one of the most economically severe viral diseases affecting grapevines worldwide. The disease can result in crop losses of up to 80% by greatly reducing fruit set, leading to small, seedless "shot berries." *Muscadinia rotundifolia*, a North American grape species, has previously been shown as a valuable source of GFLV resistance. The objective of this work is to quantify GFLV resistance in the progeny from a cross between the susceptible commercial rootstock 101-14 Mgt. and *M. rotundifolia* cv. Trayshed, and to study the inheritance of this trait. For GFLV inoculation, two-node cuttings of GFLV-infected *Vitis vinifera* cv. Cabernet Sauvignon were grafted onto hardwood cuttings from 32 individuals of the 101-14 × Trayshed population. Five months after grafting and growing in the greenhouse, the roots of the surviving plants were assayed for GFLV using RT-qPCR. Here we present preliminary results of GFLV concentrations in different genotypes. This work provides insight into the inheritance of GFLV resistance from *M. rotundifolia* and continues our progress toward developing new rootstocks to ameliorate the effects of GFLV.

*Funding Support: California Grape Rootstock Improvement Commission*



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## Enology and Viticulture – CONTINUED

### Greenhouse Evaluation of Grapevine Leafroll Associated Virus on Different Rootstocks

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Specific strains of Grapevine Leafroll Associated Viruses (GLRaV) severely affect the graft union. Some rootstocks (Freedom and 101-14) react strongly, while others (St. George and AXR1) rarely show graft incompatibility. To understand this response, Freedom, 101-14, St. George, and AXR1 were grafted with Cabernet franc infected with isolates LR131 (GLRaV1) or LR132 (GLRaV1 co-infected with grapevine virus A). Under greenhouse conditions, green grafts of Cabernet franc infected with LR131 or LR132 had mild leafroll symptoms on St. George and AXR1. However, symptoms were severe on Freedom and 101-14, and to a greater extent, with the LR132 isolate. Significant differences in scion dry weight were found between healthy and LR132-Cabernet franc grafted on Freedom or 101-14. Moreover, LR132 markedly reduced Freedom and 101-14 graft survival rate. GLRaV-1 concentration in the graft union was similar in all graftings infected with LR131, but St. George and 101-14 had the highest and lowest levels, respectively, when infected with LR132. We also grafted under in vitro conditions in an attempt to hasten the onset of symptoms. Under these conditions, LR132 infections prevented a graft union. Healthy and LR131Cabernet franc showed similar survival rates, but LR131 infection delayed budbreak and root initiation in Freedom and 101-14 micrografts. Differences in GLRaV-1 concentration in LR131 micrografts were not significant among rootstocks. Histological observations indicated that LR132 infection delayed callus formation between scion and rootstock and limited the vascular connection between them. Obvious callus was observed in both healthy and LR131-infected micrografts regardless of rootstock and there was a strong vascular connection two months after grafting. Overall, St. George exhibited the highest tolerance, followed by AXR1. Both Freedom and 101-14 were very sensitive. miRNA profiles are being studied to evaluate the different green graft combinations.

*Funding Support: California Grape Rootstock Improvement Commission*

### Characterizing Grapevine Powdery Mildew Resistance Genes from the Chinese Species *Vitis piasezkii*

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The cultivated grape species *Vitis vinifera* is highly susceptible to powdery mildew (PM), caused by the fungal pathogen *Erysiphe necator.* One of the primary objectives of grape breeders is to identify and breed natural sources of PM resistance into cultivated grapevine to mitigate the costs of PM management and promote health and environmental benefits. In this project, we aim to develop a physical map of the *Ren6* PM resistance locus, which was previously identified in the Chinese species, *V. piasezkii.* While comparing the resistance responses of the *Ren6, Ren4*,

## Enology and Viticulture – CONTINUED

and *Run1* resistance loci, we found that *Ren6* provides resistance against a broader array of fungal isolates. We propose using a map-based cloning approach to identify the candidate resistance (R) genes with the help of bacterial artificial chromosome (BAC) libraries. The physical map will help us identify putative resistance genes in the *Ren6* locus and allow us to compare the functionality of this gene(s) with PM resistance genes from different genetic backgrounds. Cloning PM resistance genes will help us understand different plant-pathogen gene interactions and may allow combination of PM resistance genes with different functionality to produce more durable resistance.

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### Evaluation of Resistance in *PdR1* Locus against a Hypervirulent *Xylella fastidosa* Strain

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Pierce's disease (PD) is caused by the xylem-limited bacteria *Xylella fastidiosa* (*Xf*), and is a chronic problem for viticulture in the southern United States and Mexico. PD causes an economic loss of more than $100 million every year just in California. The PD Resistance Grapevine Breeding Program discovered *PdR1*, a single dominant locus found in a resistant accession from Monterrey, Mexico. The *PdR1* locus has been introgressed successfully into different *Vitis vinifera* varieties while maintaining strong resistance to common wild types of *X. fastidiosa*. A mutated strain of *Xf* Temecula 1 was recently developed in Dr. Abhaya Dandekar's lab to study its secreted virulence factors. This hypervirulent strain (*prtA*$^-$) exhibits reduced cell length and hypermobility in grapevines, leading to early onset of PD symptoms. However, it is currently unknown how grapevines with the *PdR1* locus will respond to this strain. In this work, we investigate the effects of the *prtA*$^-$ strain in highly resistant accessions carrying the *PdR1* locus. We are phenotyping at three different time points using the cane maturation index (CMI) and leaf scorch-leaf loss (LS-LL) index. We are also quantifying bacterial titers using ELISA and quantitative-PCR from cane tissue. These results will provide insight into the mechanism of action of *PdR1* and its ability to defend against the *Xf prtA*$^-$ strain.

*Funding Support: CDFA PD/GWSS Board*



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## Enology and Viticulture – CONTINUED

### Effects of Grapevine Red Blotch Virus (GRBV) on Grape Development and Harvested Fruit

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Since its identification in 2011, grapevine red blotch disease has been widespread in the United States. This disease is caused by grapevine red blotch virus (GRBV) infection of grapevines. Over the past four years, we investigated the effects of GRBV on grape development and at harvest across multiple vineyards and varieties. In 2017, we completed a multi-year study evaluating the effects on Merlot (ME) and Cabernet Sauvignon (CS) at two locations. In addition, the CS grapevines were grafted on to two different rootstocks, 110R and 420A, allowing evaluation of rootstock effects. In previous years of the study, it was found that there were considerable differences in sugar accumulation at harvest; thus, sequential harvesting of the diseased grapes was implemented since 2016, meaning that the grapes were harvested at a later date when the sugar content had reached a similar value to the healthy grapes. Basic chemical data and total anthocyanin content were collected from veraison to harvest. Additionally, phenolic profiles of the diseased (at both harvest times) and healthy grapes at harvest were determined using a protein precipitation assay and RP-HPLC, and volatile profiles were analyzed using HS-SPME-GC-MS. Diseased grapes consistently had slower sugar accumulation, higher titratable acidity, and lower pH. The diseased grapes from CS 420A and ME were lower in total phenolics, anthocyanins, and tannins than healthy grapes. The second harvest berries had increased levels of all three when compared to the first harvest diseased berries. On the other hand, for CS 110R, the healthy fruit was lower in total phenolics, anthocyanins, and tannins than the diseased berries at both harvest dates, showing variable influences the virus may have on various rootstocks.

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## Enology and Viticulture – CONTINUED

### Precision Midseason Yield Estimation of Lake Erie Concord

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Accurately forecasting crop in vineyards is encumbered by inconsistencies in yield, both in space and time, due to environmental and management factors. Existing protocols for crop estimation do not typically involve analysis of spatial or temporal yield variation. Rather, manual measurements of one or more yield components covering a percentage of a vineyard's area are typically extrapolated to the entire area and converted into an estimated harvest mass based on historic averages. The objective of this research was to develop and evaluate a midseason crop estimation protocol that accounts for within-field variability in yield potential by incorporating stratified sampling and computation of yield estimates within management classes. We compared the midseason crop estimation protocol typically employed by the Lake Erie grape production industry (*single-mean*) with a protocol we developed that integrates precision viticulture technologies (*classified*) and replicated our work in three commercial vineyards in the 2017 season. The *single-mean* method calculated a single yield prediction for an entire vineyard based on random sampling of clusters at 30 days postbloom, when berries typically reach half of their final mass. The *classified* method divided a vineyard into management classes based on k-means clustering of spatial canopy reflectance data collected from budbreak to after bloom. Stratified random sampling was used to make yield predictions in each management class. The *classified* predictions were within 7% of final yield in all three commercial vineyards where these protocols were tested. The traditional *single-mean* predictions had up to 20% greater prediction error than the *classified* predictions when compared with actual yield. These prediction errors can be attributed partially to fluctuations in berry weight during the harvest window and may be reduced as future research augments this crop estimation protocol with the addition of a predictive berry weight model currently under development.

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WED/THUR POSTER ABSTRACTS

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## Enology and Viticulture – CONTINUED

### Identification of Anthocyanins in Enchantment Grapes and during Wine Production

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The Enchantment winegrape is a new *Vitis* hybrid cultivar developed by the University of Arkansas System Division of Agriculture. Enchantment includes in its parentage the *V. vinifera* cultivars Petit Syrah, Alicante Bouschet, and Petit Bouschet and the hybrid cultivar Salvador. This teinturier red winegrape has shown consistent good production in Arkansas and similar growing regions. Preliminary work on the winemaking potential of Enchantment demonstrated that the wine has a deep, red color and *vinifera*-like sensory characteristics. This research identified anthocyanins in berries and during wine production of Enchantment harvested in 2017. Grapes were destemmed, crushed, and fermented on the skins for four days. Berry samples were taken prior to crush and wine fermentation samples were taken at 8, 16, 24, and 32 days. Berries were separated into skins, seeds, and flesh, where skins had the highest total anthocyanins (1165.3 mg/100 g fresh weight), followed by flesh (10.5 mg/100 g), while seeds contained no anthocyanins. In the berries and wines, only anthocyanin-3-glucosides and their acetyl and coumaric acid derivatives were present. This is a significant finding, as native cultivars typically contain more anthocyanin-3,5-diglucosides, which exhibit less color stability. Malvidin-3-glucoside was the predominant anthocyanin in berry skins, flesh and wines, and other anthocyanin-3-glucosides included delphinidin, cyanidin, petunidin, and peonidin. During fermentation, total and individual anthocyanin concentrations increased between eight (100.4 mg/100 mL total anthocyanins) and 16 days (138.2 mg/100 mL total anthocyanins), and then decreased, likely due to complex formation with tannins. This study demonstrated that Enchantment grapes and wine have a *vinifera*-like anthocyanin profile, with malvidin-3-glucoside as the predominant anthocyanin. Therefore, wine produced from Enchantment grapes will likely have more depth of color and greater color stability than wine produced from other native cultivars grown in Arkansas and the surrounding region.

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## Enology and Viticulture – CONTINUED

### Genotype and Environment Effects on Polyphenol Profile and Coloration of Grape Berries in Nebraska

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The vineyard and winery industry in Nebraska has grown greatly in the past 20 years. Since 1998, studies on cultivar evaluation of climate effects have been established to choose appropriate cultivars for specific regions of Nebraska. Although many studies have been performed to determine the impact of environmental factors on flavonoid accumulation in grape, little information is available for cultivars grown in Nebraska. Therefore, this study monitored the polyphenol accumulation, especially those responsible for berry color and antioxidant activities, such as anthocyanins and flavonoids, under darkness, UV light, jasmonic acid, or abscisic acid treatments for major grape cultivars in Nebraska. Grape berries of Frontenac, Frontenac gris, Norton, and Edelweiss were collected at veraison, surface disinfested, and cultured on 0.7% agar without pedicels. After maturity, the berries were collected for characteristic analysis. Skins and seeds were then separated and used for polyphenol profile and antioxidant activity analysis. In general, berry weight, Brix, and pH were observed to increase after the veraison stage. The effects of the darkness, UV, jasmonic acid, and abscisic acid treatments on the polyphenol profile and antioxidant activity were cultivar-dependent. For each treatment, optimal conditions for polyphenol accumulation were identified. In general, the polyphenol compounds increased with UV light and jasmonic acid treatments but decreased with darkness and abscisic acid treatments. However, skin polyphenols accumulated during berry development, which was different from that in seeds. Moreover, total anthocyanins in skins of red color cultivars increased during berry development. Anthocyanins in berries cultured in agar were less abundant than in berries collected from the field at veraison and ripeness. This study investigated several factors which influence the secondary metabolites of grapes. These findings would be useful for producing grapes rich in health-beneficial polyphenols.

*Funding Support: UNL-IANR funds*



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## Enology and Viticulture – CONTINUED

### Influence of Rootstock on Scion Mortality, Performance, and Berry Composition in the Four Corners Region of New Mexico

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Rootstock choice has been shown to influence scion phenological development, leaf senescence, timing of dormancy, vegetative growth, and berry maturation. Our research aim was to determine and describe the rootstock influence on scion mortality, vine development and vegetative growth, yield components, and berry composition in the high-elevation (>1700 m), semi-arid climate of northwest New Mexico. Nine different rootstocks in grafted combinations with Refosco and Gewürztraminer were field-planted in 2008 in a completely randomized design with six replications and four vines per plot. After nine years, Refosco vines grafted to 775 Paulsen (*Vitis berlandieri* × *V. rupestris*) had the lowest vine mortality (25%), whereas among Gewürztraminer vines, those grafted to 775 Paulsen had the greatest crop weight. Gewürztraminer vines grafted onto SO4 (*V. berlandieri* × *V. riparia*) exhibited the lowest vine mortality (13%), and Refosco vines grafted to SO4 had the greatest crop weight (8.25 kg/vine) relative to all other tested scion/rootstock combinations. The earliest berry maturity and greatest YAN (yeast assimilable nitrogen) was associated with Gewürztraminer vines grafted to 1103 Paulsen. Lowest crop weights were associated with vines grafted to either 110 Richter (*V. berlandieri* × *V. rupestris*) or 5C (*V. berlandieri* × *V. riparia*). Understanding how rootstock selection can impact scion performance in non-traditional winegrowing regions is of immediate practical importance to local growers and can provide insight by comparison to data from more established regions.

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### Developing a Model System to Identify Main Mechanisms Involved in Nitrogen Growth Responses of Grafted Grapevines

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The scion growth potential (vigor) of grafted grapevines results from the three-way interaction between environment, scion genotype, and rootstock genotype. Since nitrogen (N) availability is a major driver of grapevine growth, understanding N regulation in scion and rootstock will lead to new insights to control canopy size in vineyards. We are developing a model system to study N regulation by evaluating the N supply responses of 12 scion-rootstock combinations with known differences in scion and rootstock vigor. Our primary objectives are to understand the influence of scions and rootstocks on growth parameters and resource allocation and to evaluate the role of N uptake regulation in scion growth response. To address the first objective, we measured components of vine water relations and gas exchange, plant biomass, carbon (C), and N allocation in four plants tissues (leaves, stem, trunk, and roots). Preliminary results supported the expected vigor behavior of the

three Pinot noir scions used in this experiment, but this was not true for the four rootstocks examined. N availability altered C and N allocation in all tissues, but scion vigor was not affected. The N requirement for one-year old vines was satisfied by our lowest N rate, and the experiment will be repeated under greater N limitation. However, this first trial will allow us to study the role of C and N reserves on scion vigor during the second growing season. We are addressing the second objective by comparing N uptake and N transport between two rootstocks using $_{15}NO_3$. Several experiments are underway to compare N uptake kinetics over a range of N concentrations and N transport rate in response to plant N status. These analyses will be complemented with gene expression studies targeting N transport and signaling in roots and leaves.

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## Performance of Rootstocks in a Cabernet Sauvignon Vineyard Infected with Grapevine Fanleaf Virus

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A rootstock trial was planted in the Alexander Valley in Sonoma County, CA, in 2012, one year after the previous vineyard on 3309C rootstock was removed due to yield loss caused by grapevine fanleaf virus (GFLV). The dagger nematode *Xiphinema index* was present in soil samples collected in the vineyard. The planting site remained fallow for one year, the trellis system was left in place, and no preplanting nematicide applications were made. Eleven rootstock treatments were planted as green benchgrafts in a randomized complete block design with eight replications of five-vine plots. The vines were trained to bi-lateral cordons and spur pruned. The rootstocks evaluated were GRN 1, GRN 2, GRN 3, GRN 4, GRN 5, and O39-16, which are resistant to *X. index* and RS 3, RS 9, Scwharzmann, 1616C, and 1103P, which have medium or low resistance. Yields were collected in 2016 and vine growth parameters in 2015 to 2017. Pruning weights consistently increased for nearly all rootstocks. GRN 2 and 039-16 tended to be the largest vines and GRN 1, the smallest of the GRN stocks. RS 3, RS 9, and 1616C were the smallest vines in all years, with pruning weights of 0.5 kg/vine in 2016. Vines on O39-16 had the largest yield in 2016 at 9.3 kg/vine, followed by GRN 1 with 7.8 kg/vine. Vines on RS 3, RS 9, and 1616C were among the lowest yielding: in 2016, RS 3 and RS 9 yielded 5.2 and 3.7 kg/vine, respectively. GRN 1 had a crop load of 5.3, the highest of the GRN stocks, while the crop loads of other GRN stocks and O39-16 ranged from 2.6 to 3.3. RS 9 and RS 3 had excessive crop loads of eight and 9.9, respectively, due to extremely low pruning weights.

*Funding Support: No external funding*



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## Enology and Viticulture – CONTINUED

### Effects of Cold Temperature Exposure Regimes on Damage to Phloem and Buds of Dormant Merlot Canes

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The effects of cold temperature exposure regimes on phloem freeze injury and subsequent bud break and shoot development were determined using dormant Merlot canes. Freshly pruned canes were exposed to cold temperature treatments in a walk-in freezer programed to cool at -2°C/hr, while bud and phloem hardiness was determined using a standard differential thermal analysis protocol. In one experiment, bundles of 13 canes were removed during the cooling ramp-down at set temperatures between -12 and -28°C. In another experiment, after the ramp-down, the freezer temperature was held constant at ~ 4°C above the temperature lethal to 50% of buds, during which bundles of 10 canes were removed after 0, 1, 4, 8, and 24 hrs. In both experiments, after the exposure treatments ~half of the canes were sectioned and assessed visually for bud and phloem damage, and the rest were cultured to initiate root and shoot development. Results indicate that budbreak and shoot development were not affected until phloem damage exceeded 80%, which occurred around the same temperature as that causing 50% primary bud mortality. The amount of bud and phloem damage increased with the duration of exposure to 4°C above the 50% bud-lethal temperature. More than 80% of phloem was damaged after 8 hr, and 50% of buds were killed after 24 hr exposure.

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

### Pruning Approaches to Revive Cold-Injured Grapevines

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In Washington State, some of the older vineyards have experienced repeated cold injury, reducing their productivity. During 2014 to 2016, a field trial was conducted in an own-rooted Merlot vineyard planted in 1981 to test the potential of various pruning methods to revive cold-injured vines. Treatments were: 1) standard spur pruning (control); 2) modified kicker cane – 3 short canes were trained onto the cordon while the remainder of the cordon was disbudded; 3) disbudding – all buds were removed from the cordon; and 4) chopped cordon – the cordon was removed by cutting the trunk 10 to 15 cm below the cordon wire, and one strong sucker was trained onto the wire to reestablish the cordon. Vine recovery was measured through 2016 by determining pruning weight, yield components, and fruit composition. In 2014, shoot growth and periderm formation were the highest in chopped cordon, indicating the fastest vegetative recovery, albeit with no crop. The three-year average yield (3.9 kg/vine) and cumulative yield (11.8 kg/vine) in kicker cane was significantly higher than in other treatments. Cluster numbers were higher, but cluster weights were lower in kicker cane in 2016. Pruning weight measurements indicated that kicker cane had higher vigor compared to standard spur and disbudding. In 2016, the yield to pruning weight ratio for kicker cane

(6.3) was in the recommended range (five to 10), while for other treatments, it was under five. Overall fruit composition remained similar in all four treatments during all three years, except for a slight increase or decrease in soluble solids, titratable acidity, or pH, only in one year. Observations indicate that chopped cordon promoted greater uniformity, but kicker cane offered higher economic returns during the three study years.

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## Changes in Grape Flavonoid Composition Driven by Exposure to Solar Radiation: New View on an Old Topic

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Cultural practices such as leaf removal or shoot thinning are used to expose grapes to solar radiation. Thus, there are many studies suggesting that solar radiation may promote flavonoid biosynthesis through transcriptomic regulation. However, as exposure increases there is a growing need to cope with direct damage from incident radiation and elevated berry temperature, which can result in flavonoid synthesis inhibition and degradation. Flavonoids are very effective at absorbing harmful wavelengths when located in the epidermis, but they are also effective free radical scavengers, protecting sensitive cell components such as genetic material, photosynthetic apparatus, or enzymatic machinery. We used a fish-eye lens from the perspective of the grapes to assess canopy porosity at the berry scale. In addition, samples were collected to characterize the effects of exposure at the cluster, berry, and half berry scale. Each of the four flavonoid groups measured by HPLC, anthocyanins, flavonols, flavan-3-ols, and proanthocyanidins, showed a different dose-response relationship to berry exposure. This approach allowed us to see clearly three phases in their response: I) increased concentration, resulting from a predominant increase in biosynthesis; II) an optimal peak, resulting from a compensation point between synthesis and degradation; and III) decreased concentration, resulting from a higher rate of degradation compared to their synthesis biosynthesis. For instance, anthocyanins, which are highly desirable in red full-bodied wines, showed a very low compensation point between synthesis and degradation (<20% of canopy porosity). On the other hand, flavonols, which have a predominant role in mitigating excess radiation, had a much higher compensation point. These results suggest a need to revise the relationships between solar radiation and flavonoid synthesis induction, as this could make winegrape producers reconsider cultural practices in locations were light is not limiting.

*Funding Support: Oakville Experimental Vineyard*



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## Enology and Viticulture – CONTINUED

### The Impact of Leaf Removal on Anthocyanin, Quercetin, and Procyanidin Composition in *Vitis vinifera* L. Syrah Grapes

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Leaf removal is a canopy management practice that can improve wine quality and influence the physical attributes of clusters. Prebloom leaf removal affects cluster size, compactness, and yield while improving quality, while leaf removal at fruit set or veraison does not alter physical attributes, but does impact quality. Phenolic compounds important to wine quality are significantly affected by leaf removal. The effects of seven leaf removal timing and intensity treatments on anthocyanins, procyanidins, and quercetins were evaluated over three years in Syrah grapes grown in Osoyoos, British Columbia. Prebloom leaf removal was applied by removing either four or six basal leaves at two weeks prior to bloom. Fruit set leaf removal was applied by removing 50 or 100% of leaves in the fruiting zone, and similarly for the veraison treatment, 50 or 100% of leaves in the fruiting zone were removed. HPLC analysis of phenolic components in grape skin and seeds included the quercetin glycosides, anthocyanins, catechin, and epicatechin. The prebloom treatments reduced catechin and epicatechin by up to 40% in both seeds and skin, while the other treatments also resulted in some reduction. Quercetin-3-glucoside and quercetin-3-glucuronide increased with most treatments and by up to 70% in the six leaf prebloom treatment. Total anthocyanins increased with prebloom leaf removal in two out of three years, but in fruit set and veraison treatments, they decreased or were not significantly different. Non-acylated anthocyanins were affected most by leaf removal, while the acetylglucosides and cinnamoyl derivatives were not different. The results show that timing and intensity of leaf removal can alter fruit quality through changes in phenolic composition.

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

### Anthocyanin Regulation Under Deficit Irrigation and its Role in Improving the Red Color of Scarlet Royal Table Grapes

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Deficit irrigation (DI) is an irrigation scheduling technique used to improve red color development in grapes. The red color in grapes is mainly due to the plant pigment anthocyanin. Anthocyanin biosynthesis was investigated in Scarlet Royal grapes, grown in the San Joaquin and Coachella Valleys and subjected to two different deficit irrigation strategies. Total berry skin anthocyanin contents and the individual pigment compounds increased with DI at both experimental sites. DI induced expression of several genes involved in anthocyanin accumulation. Expression analysis of genes involved in anthocyanin biosynthesis revealed the induction of key genes such as UDP-glucose: flavonoid-3-O-glucosyltransferase (UFGT) following DI. However, the expression of this gene was lower in the Coachella Valley than in the San Joaquin Valley. Data also showed an increase in the expression of chalcone

synthase gene (CHS2) in response to DI treatments at both sites; however, the expression of this gene was higher in Coachella Valley after ending the deficit treatment and re-irrigating the vines. This gene is well known to be involved in response to different stress conditions, besides its role in anthocyanin biosynthesis. Thus, the high expression of this gene in the Coachella Valley could be due to the induction by other environmental stress conditions. This data was supported by the fact that antioxidant genes expression showed a lower level in the Coachella Valley. Together, these findings suggest that the lack of red coloration in the Coachella Valley could be due partially to less antioxidant activity. The low level of the antioxidant activity allows the accumulation of free radicals, resulting in impairing anthocyanin biosynthesis.

*Funding Support: California Table Grape Commission*

## Evaluation of Plant-Based Measurement as an Irrigation Scheduling Tool in Grapevine

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A more time-efficient, less intensive, plant-based method that diversifies the irrigation scheduling toolkit is needed for irrigation scheduling. A portable leaf porometer measuring stomatal conductance to water vapor provides rapid, real-time, nondestructive data on current vine-water status, making it an appealing tool for irrigation scheduling. Three irrigation treatments were applied to a red (Merlot) and white (Chardonnay) cultivar of field grown grapevines (*Vitis vinifera* L.) over two growing seasons to evaluate the efficacy of porometry in determining the extent of water stress. These treatments included high irrigation (~100% $ET_c$), moderate irrigation (~50% $ET_c$), and low irrigation (~25% $ET_c$). From full bloom through physiological maturity of grape berries, stomatal conductance and midday leaf water potential were recorded concurrently from a single leaf in each replication four to six days after the most recent irrigation. A significant positive linear relationship was observed between midday leaf water potential and stomatal conductance in all treatments of both cultivars. This indicates that stomatal conductance measured by a porometer can be used to detect water status in grapevines and has potential as a tool for scheduling irrigation.

*Funding Support: Northwest Center for Small Fruit Research (USDA)*

## Assessing Spatial Variability of Grape Skin Flavonoids at the Vineyard Scale Based on Plant Water Status Mapping

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Plant water stress affects grape (*Vitis vinifera* L. cv. Cabernet Sauvignon) berry composition and is variable in space due to variations in the physical environment at the growing site. We monitored the natural variability of grapevine water stress using stem water potential ($\Psi_{stem}$) and leaf gas exchange in an equidistant grid in a commercial vineyard. Spatial differences were measured and related to topo-



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graphical variation by modeling. Geospatial analysis and clustering allowed us to differentiate the vineyard block into two distinct zones with severe or moderate water stress that varied by 0.2 MPa. Differences in stem water potential affected stomatal conductance, net carbon assimilation, and intrinsic water use efficiency, all of which were different on all measurement dates. The two zones were sampled selectively at harvest for measurements of berry chemistry. Water status zone did not affect berry mass or yield per vine. There was significant difference in total soluble solids (3.56 Brix) and titratable acidity, indicating a direct effect of water stress on ripening acceleration. Berry skin flavonol and anthocyanin composition and concentration were measured by C18 reversed-phased high-performance liquid chromatography (HPLC). The anthocyanins were most affected by the two water stress zones. Dihydroxylated anthocyanins were more affected than trihydroxylated; therefore, the ratio of the two forms increased. Flavonols were different in total amounts, but hydroxylation patterns were not affected. Proanthocyanidin isolates were characterized by acid catalysis in the presence of excess phloroglucinol, followed by reversed-phase HPLC. Proanthocyanidins showed the least significant difference, although (+)-catechin terminal subunits were important predictors in a partial least square model used to summarize the multivariate relationships, predicting $\Psi_{stem}$ or the management zone. The results provide fundamental information on vineyard water status that could discriminate harvest or direct vineyard operators to modify irrigation management to equilibrate berry composition at harvest.

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## Differences in Spatial Water Uptake by Rootstocks 110R and 101-14Mg Revealed by Multidimensional ERT

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A proximal sensing method was developed as a novel noninvasive method (electrical resistivity tomography, ERT) to compare dynamic changes in root growth and water uptake by rootstocks used in vineyards. An experiment was conducted with three replicates of four plants of 110R and 101-14 MG onto which Chardonnay was grafted, which were not irrigated during the 2017 field season to reach a severe water stress status. Primary metabolism was characterized by predawn and stem water potentials throughout the day, carbon isotopic discrimination of sugars ($dC_{13}$), and stomatal conductance and net leaf carbon assimilation were also measured. Plant vigor and nutrition were assessed by pruning weights and mineral nutrient analysis, respectively. During the dormant season, three soil pits were opened in each experimental unit to analyze the soil and install TDR sensors. At the end of season, roots were sampled, separated from the soil, and weighed, then ERT in 2D and 3D was performed. ERT was corrected by the temperature and reported to a standard temperature of 25°C, then two different pedoelectrical models were fitted. The Archie model performed better and allowed transformation of electrical resistivity into soil volumetric water with an error of 1.2% volune ($R^2$ = 0.73). The first 3D images of water distribution under grapevine were obtained, and up to one-fold of spatial differences in the quantity of water absorbed between grapevines

was measured in the two rootstocks. Spatial distribution of water correlated significantly to predawn water potential in both rootstocks. 110R and 101-14Mg showed contrasting water uptake behavior that was related to greater pruning mass and water stress that were significantly different between rootstocks. Water distribution correlated with the presence of roots and was used to develop the first electrical image of the root distribution of a grapevine in situ.

*Funding Support: American Vineyard Foundation*

### $\delta^{13}C$ in Sugars as a Proxy for Photosynthesis and Water Stress in Precision Viticulture: A Statewide Study in California

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Measurement of carbon isotopic discrimination of berry sugars at harvest ($\delta^{13}C$) is an integrated assessment of water status during grape ripening. It is an alternative to traditional measurements of water status, crucial for understanding spatial variability of plant physiology at the vineyard scale. This work was performed in 91 experimental units at four different locations across California, more than 400 km apart, planted to three different table and winegrape cultivars (Cabernet Sauvignon, Merlot, and Crimson Seedless) whose behavior ranges from isohydric to anisohydric and in between, as reported by recent scientific literature. Leaf physiology (photosynthesis and stomatal conductance) and stem water potential were routinely measured in each experimental unit. $\delta^{13}C$ was measured at harvest and a direct relationship was evident between stem water potential ($R^2 = 0.72$), stomatal conductance ($R^2 = 0.66$), and net carbon assimilation ($R^2 = 0.62$) measured throughout the season. All cultivars were pooled together and behaved the same, independently from their reported hydric behavior. This was confirmed by crossed relationships between stem water potential, stomatal conductance, net carbon assimilation, and crop water stress index that could not discriminate among the reported hydric behaviors. A unique state-wide calibration was therefore developed between $\delta^{13}C$ and plant water status. The use of $\delta^{13}C$ was therefore tested in a precision viticulture context and measured on grapes sampled on a spatially equidistant grid in a 3.5 ha field where stem water potentials were also measured throughout the field season. The two management zones obtained by $\delta^{13}C$ and stem water potential were spatially similar at 72% and allowed to separate the harvest into two pools, with statistically different grape composition (soluble solids, organic acids, and anthocyanin profiles). Our results provided evidence that $d^{13}C$ discrimination is a reliable and repeatable assessor of plant water status and whole plant physiology in vineyard ecosystems.

*Funding Support: USDA-NIFA*



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## Enology and Viticulture – CONTINUED

### Partial Solar Radiation Exclusion, Not Applied Water Amount, Mitigates Grape Berry Flavonoid Concentration

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Increased daytime temperatures and intermittent heat spikes during engustment negatively affect the biosynthesis, retention, and degradation of flavonoids in grape berries. There is little information on how to mitigate radiation damage and degradation that may occur under these conditions. A field experiment was conducted with Cabernet Sauvignon/110R using one-meter, black, polyethylene shade nets with 40% light transmissivity to determine the effects of partial solar radiation exclusion and applied water amounts on the productivity and primary and secondary metabolites of grapevine berry. One netting treatment of 40% black net and one untreated control with no net were applied shortly after fruit set at E-L 29 and retained until harvest. Two applied water amounts of 0.65 crop evapotranspiration (ETc, control) and 1.3 ETc, were applied. At harvest, there was no effect of treatments on pH or titratable acidity. However, individual berry mass was greater in controls than in shade net treatments. Diurnal cluster temperature shifts on both sides of the canopy were recorded. During peak daytime temperatures, cluster temperatures were 3.9°C greater in the control, although no effect was attributed to applied water amounts. Cluster damage attributed to solar radiation exposure was quantified. Although yield was unchanged, damaged cluster count and weight were significantly greater in treatments without shade netting applied. Anthocyanins and flavonols of berry skins were measured using reversed-phase HPLC. At harvest, treatments without shade nets had higher flavonol concentrations but a lower tri/di-hydroxylation ratio than shaded treatments. However, anthocyanin concentration was highest in shaded berries. Our results provide evidence that regardless of applied water amounts in a vineyard, effective shade netting may alleviate berry stress from excessive solar radiation exposure. It may do so without greatly affecting yield at harvest and can mitigate the amount of visible radiation damage on the fruit.

*Funding Support: Oakville Experimental Vineyard*

### Cabernet Sauvignon Berry Quality from Vines Irrigated through Direct Root Zone Irrigation

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Water availability is a growing concern as the wine industry continues to expand. Washington irrigation sources are dwindling, but with efficient, water conserving irrigation, the amount of water used in vineyards can be reduced while producing high-quality winegrapes. Cabernet Sauvignon grape vines at Kiona Vineyards in the Red Mountain AVA were subjected to direct root zone (DRZ) irrigation during the 2017 growing season. Water was delivered directly to the lower root zone of each vine at 0 and 2-foot depths through one-inch diameter PVC piping at 80, 60, and 40% of commercial irrigation. DRZ irrigation remained at a commercial

rate for the beginning of the growing season until fruit set, when water application rates were reduced to specified percentages for the remainder of the growing season. With DRZ irrigation, no significant differences were observed in pH, tannin concentration, anthocyanin concentration, or Brix. Berry diameter was reduced as water application was reduced. Further experimentation with these water rates and irrigation depth will be done to understand the long-term effects of water stress on the productivity of the vines.

*Funding Support: Washington Grape and Wine Research Program, Northwest Center for Small Fruit Research, Washington State Concord Grape Research Council, WSDA, and Western Sustainable Agriculture Research and Education*

## Conserving Water While Increasing Efficiency of Grape Production through Direct Root-Zone (DRZ) Deficit Irrigation

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Increased crop water use efficiency of winegrapes was achieved through DRZ drip irrigation during a three-year study near Benton City, WA. Rather than using buried lines to deliver subsurface drip irrigation, DRZ is applied through vertical PVC delivery tubes inserted into the soil 0.5 m either side of the trunk and in line with the trellis system. DRZ allowed water to be applied into the lower root-zone at 30, 60, or 90 cm depths. This technique saved at least 40% of the water used by surface drip irrigation and yielded from 70 to 95% of commercial production. These results confirm our hypothesis that DRZ deficit irrigation delivery can conserve water while enhancing use of water to regulate vine activity to achieve grape quality and quantity goals. DRZ was initiated at fruit set and maintained on the same schedule as commercial surface drip irrigation through harvest. Field and greenhouse observations using mini-rhizotrons verified that own-rooted winegrapes (Cabernet Sauvignon) developed the mass of roots near and below the depth of water delivery in the soil profile. Results to date have not documented a particular advantage for a specific depth of delivery or for pulsed water delivery rather than continuous flow during irrigation sets. During the 2017 growing seaons, vines receiving DRZ delivery demonstrated greater photosynthetic capacity, as measured by rates of stomatal conductance and $CO_2$ assimilation, than vines irrigated at equal rates by surface drip. Quality of grapes, measured as higher Brix, tannins, anthocyanin, and lower acidity, increased in direct proportion with increasing rates of deficit irrigation and resulting plant water stress. All irrigation events and soil moisture content were tracked with recording electric capacitance probes, and actual water delivery was quantified with mechanical meters read after irrigation sets.

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## Enology and Viticulture – CONTINUED

### Interaction of Deficit Irrigation and Grapevine Red Blotch Virus (GRBV) on Disease Development and Grapevine Physiology

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While moderate water deficits advance ripening and improve fruit quality in healthy grapevines, they can potentially amplify negative effects of viral disease in GRBV-infected grapevines. Therefore, a field experiment with two irrigation treatments, wet and dry, and two disease statuses, healthy (RB-) and infected (RB+), was initiated to understand the interaction between GRBV infection and deficit irrigation. Wet vines were irrigated at 100% of crop evapotranspiration ($ET_c$), while dry vines received water at 66% $ET_c$. Healthy and infected vines were confirmed by PCR-based assays. Disease progression and severity were recorded weekly after first symptoms were observed on RB+ vines, and vine water status ($\Psi_{stem}$) was regularly monitored throughout the growing season. At harvest, yield and yield components were determined, and berry samples were collected for compositional analyses. There was no significant interaction between irrigation treatment and disease status on disease progression and severity. Preveraison $\Psi_{stem}$ was not affected by disease status, but was significantly higher in RB+ vines postveraison. The higher $\Psi_{stem}$ in RB+ vines resulted in larger berries and yield at harvest, but few of the differences in yield and yield components among treatments were significant. Berry flavonoids were more strongly affected by disease status than sugars and acids, with little effect of irrigation treatment. In skins and seeds, significant differences among treatments were observed in the concentration and content of anthocyanins and iron-reactive phenolics (IRPs) but not tannins. Small differences in tannins coupled with large differences in IRPs suggests that GRBV strongly inhibited biosynthesis of nontannin IRPs, particularly in seeds. Taken together, these results suggest that keeping vines well-watered may mitigate some of the negative effects of GRBV, but ultimate changes in secondary metabolism due to GRBV infection may necessitate using infected fruit for different wine programs or for blending with lots from healthy vineyards.

*Funding Support: American Vineyard Foundation*

### Exogenous Application of Abscisic Acid (s-ABA) Does Not Influence Fruit Ripening in Red Blotch-Infected Grapevines

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Plant growth regulators are commonly used to improve ripening and berry composition in healthy grapevines. Recently, it has been suggested that grapevine red blotch virus (GRBV) disrupts the normal hormonal signaling involved in ripening onset in berries. Since endogenously produced abscisic acid (s-ABA) plays a large role in berry ripening, it is possible that exogenous applications of s-ABA could mitigate the deleterious effects of GRBV. Therefore, the effects of exogenous s-ABA application on fruit ripening in GRBV-infected grapevines were tested in two Oregon AVAs characterized by different climates: the Willamette Valley (WV; cool and wet) and the Rogue Valley (RV; warm and dry). At each site, candidate vines were identified

based on previous symptoms and confirmed for GRBV infection with PCR-based assays. Cluster-directed spray applications of s-ABA were made at 50% veraison and 10 to 14 days later at a rate of 300 mg/L. Experimental treatments were a 2 × 2 factorial combination of disease condition: healthy (RB-) and infected (RB+), and s-ABA application: spray (ABA+) or no spray (ABA-). At harvest, there were no significant effects of s-ABA on berry fresh weight, total soluble solids (TSS), pH, or titratable acidity within RB+ or RB- vines. There were also no significant effects of disease status on the aforementioned parameters in WV vines, and in RV vines, only TSS was significantly higher in RB- vines. There were no significant interactions between s-ABA application and disease status with respect to polyphenolic composition in skins and seeds. Surprisingly, ABA+ vines had lower concentrations of tannins and iron-reactive phenolics across either disease status in the skins, but since there were minimal effects in the seeds, there were nonsignificant treatment effects in total concentration. Overall, exogenous application of s-ABA at veraison did not improve fruit composition across two distinct growing regions.

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## Postveraison Water Deficits Improve Pinot noir Fruit Quality without a Yield Penalty

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Despite increasing Pinot noir acreage in warmer and more arid growing regions, cultivar-specific drought responses remain poorly described in the literature. A multi-year field experiment was established with eight irrigation treatments designed to alter vine water status either pre- or postveraison. Irrigation was scheduled based on applying water at fractions of estimated crop evapotranspiration ($ET_c$) ranging from 25 to 100%. Vine water status was monitored with regular measurements of midday stem water potential ($\Psi_{stem}$) throughout the growing season. At harvest, fruit were analyzed for yield and quality characteristics. The treatments significantly altered vine water status both pre- and postveraison, giving rise to four levels of water stress at both times. Berry size correlated negatively with water deficits at both times. Berry primary metabolism (Brix, pH, and TA) was less responsive to water deficits than secondary metabolism (polyphenolics). Total anthocyanins increased with water deficit both pre- and postveraison. The response was more sensitive preveraison, but the differences were not significant across treatments. In contrast, tannins and iron-reactive phenolics (IRPs) in skins and seeds were significantly impacted by the treatments. Skin tannins and IRPs increased with preveraison water deficits but decreased with postveraison water deficits. Seed tannins and IRPs increased with preveraison water deficits but were not affected by postveraison water deficits. In general, berry secondary metabolism was more sensitive to preveraison water deficits. However, postveraison water deficits resulted in higher concentrations of secondary metabolites overall. While wine sensory analyses have yet to be completed, the results suggest that postveraison water deficits may be more effective at improving Pinot noir fruit quality without a yield penalty than preveraison deficits.

*Funding Support: Oregon Wine Board*



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## Re-evaluating Field Methods of Water Status Determination in the Vineyard

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The pressure chamber is a widely used tool for assessing water potential (Ψ) in plants and is commonly used as a tool in vineyard irrigation scheduling. Although it is regarded as the most robust method to assess plant water status in the field, there continues to be disagreement among users about proper technique for determining both midday leaf ($\Psi_{leaf}$) and midday stem ($\Psi_{stem}$) water potential. To resolve these discrepancies, three experiments were performed to understand how varied techniques affect either $\Psi_{leaf}$ and $\Psi_{stem}$ values: (1) $\Psi_{leaf}$ response to the time interval between sample excision and pressurization; (2) $\Psi_{leaf}$ response to sample preparation method (e.g., petiole re-cutting) prior to pressurization; and (3) $\Psi_{stem}$ response to sample equilibration time. All experiments were performed by two operators using the same instrument. There were no significant effects of time interval or operator on $\Psi_{leaf}$ at time intervals from 15 to 60 sec. Few significant differences were found in $\Psi_{leaf}$ among sample preparation methods (experiment 2), and they depended on operator. $\Psi_{stem}$ varied 5% when samples were allowed to equilibrate from 10 to 240 min prior to determination. The results show that time intervals of up to 60 sec between excision and pressurization were acceptable for accurate data, and petiole re-cutting did not substantively affect $\Psi_{leaf}$ determination. Additionally, $\Psi_{stem}$ equilibration times can be as short as 10 min. However, significant differences were observed between operators across all three experiments. Thus, the technical skill of the operator during pressurization may play a larger role in the outcome of the determination relative to the preparation of the sample prior to pressurization.

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## Performance of Cabernet Sauvignon under Direct Root-Zone Deficit Irrigation

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Water for vineyard irrigation now faces potential limits in seasonally dry regions owing to multiple competing demands from other crops and unstable climatic patterns. Deficit irrigation has been proven effective in saving water, increasing water use efficiency while sustaining fruit yield, and enhancing grape quality for red wines. However, conventional surface deficit irrigation causes moisture loss through water evaporation and encourages weed growth, and grapevines may produce more shallow roots rather than distribute roots into deep soil, which can weaken their response to extreme drought and water shortage. Delivering a limited water supply directly into the deep root-zone by using subsurface microirrigation may be a good strategy to address these challenges. We hypothesize that limited water delivered directly into the middle and lower root-zone will help sustain grapevine growth and grape production, improve water use efficiency, and optimize biomass partitioning in grapevine. Using Cabernet Sauvignon as a model variety, we conducted both greenhouse and field experiments (2015 to 2017) to investigate the effects of direct

root-zone deficit irrigation (DRZ) with different rates, depths, and water delivery methods (constant versus pulse) on grape production, grapevine growth, and root distribution. Total irrigation amount significantly influenced fruit yield, and grape quality was enhanced under concomitant reduction in seasonal water delivery amounts. No significant differences in grape yield were attributed to any irrigation depth and method of DRZ delivery; however, irrigation depth had a significant influence on root length, number, and distribution. Compared with commercial surface drip irrigation, DRZ improved photosynthesis capacity and advanced crop water use efficiency in direct correlation with the reduced amounts of water applied. Meanwhile, rates of biomass accumulation at different growing stages were variable, which must be considered for efficient irrigation management. After three growing seasons, DRZ shows promise for water conservation and enhancement of winegrape production.

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## Applied Water Amount, Not Timing of Leaf Removal, Alters Grapevine Berry Flavonoid Content in Cabernet Sauvignon

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California viticulture is recognized for its nonlimiting source of solar radiation during the growing season. A consequence of such solar radiation is water deficit conditions. Vineyard managers must determine appropriate balances of canopy management and irrigation budgeting to produce suitable yields without compromising berry chemistry. In response, a study was designed to test the interactive effects of leaf removal timing (prebloom and post-fruit set, compared to an untreated control) and applied water amounts (1.0, 0.5, and 0.25 crop evapotranspiration replacement (ETc)) on Cabernet Sauvignon/110R in Oakville, CA. Stem water potential was lower in the 0.25 ETc, regardless of leaf removal. A 40% reduction in net carbon assimilation was evident in the 0.25 ETc treatments as well. This was mediated by a lower stomatal conductance with 0.25 ETc. There was no effect of leaf removal on components of yield, including number of berries set. The 0.25 ETc treatment reduced berry mass and yield, but 0.5 and 1.0 ETc treatments were not different from each other. There was a significant interaction of leaf removal and irrigation on pruning weight and Ravaz index. Reducing the applied water amount significantly influenced anthocyanin and proanthocyanidin content, but normalized dried skin mass (DSM), suggesting a concentration effect as opposed to an increase in biosynthesis. Leaf removal affected flavonol content, specifically kaempferol-3-*O*-glucoside in both DSM and on a per berry basis, consistent with the existing light exposure literature. Clear skies and long periods with minimal precipitation paired with severe reduction in irrigation, will have a stronger influence on berry chemistry than leaf removal practices. Our results indicated that cluster microclimate without leaf removal was already optimized. Although not as

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impactful, there still appears to be potential for understanding leaf removal influence on berry physiology and its effect on vine balance in premium regions.

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### Microtensiometer Implant for Continuous, Direct Measure of Stem Water Potential

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Vine water status determines vine growth, productivity, fruit composition, and terroir; therefore, it is important to accurately measure and control water stress. The pressure chamber is the accepted method to measure water stress, but this instrument requires an operator and can only provide limited spot data. Other instruments like soil tensiometers, capacitive sensors, or dendrometers only measure water stress tangentially and suffer from a lack of accuracy. We present our progress in developing a microtensiometer implant to directly and continuously measure stem water potential in grapevine. The microtensiometer is a miniaturized, 5 mm × 5 mm tensiometer built through microfabrication techniques. Microtensiometer probes were embedded in the main trunk of Merlot grapevines and recorded data from Sept 2017 to Feb 2018. The sensors measured the diurnal pattern of water potential, including both predawn and midday water potential, with values ranging from -3 bars to -15 bars. Sensor measurements agreed with pressure chamber readings taken over the course of two months. The sensors continued taking readings throughout the winter during vine dormancy and measured a smaller diurnal pattern during this time. Work is underway to understand how this unprecedented higher-resolution data may be useful. These results indicate that the microtensiometer implant could be a viable alternative to the pressure chamber. Here we will further present on our latest results in testing the microtensiometer at multiple vineyards during the 2018 growing season.

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### Mechanisms of Water Movement in Grapevines during Hydraulic Redistribution

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Plants can transport water from roots in wet soil to roots in dry soil. Such hydraulic redistribution is thought to occur via the xylem and root parenchyma. However, it is unknown how the phloem contributes to overall water transport during this process. Our hypothesis is that hydraulic redistribution in grapevines is in part due to water movement to the leaves via the xylem and recycling from the leaves to the roots via the phloem. This study used deuterium-labeled water ($^2H_2O$) as a tracer of water flow. Own-rooted *Vitis vinifera* L. cv. Merlot grapevines were grown in pots with a three-way split root design. One of the three compartments was irrigated with $^2H_2O$, and the other two were left to dry. The trunk in one of the dry compartments was girdled, and the other one was left intact to distinguish xylem and phloem water movement. Xylem sap and phloem sap, trunk and root tissue, and

soil samples were collected. Water from each sample was extracted via a cryogenic method and analyzed for deuterium enrichment ($\delta^2H$). Preliminary results show deuterium enrichment in both xylem and phloem sap collected from the same petioles. The $\delta^2H$ values were six times higher in root tissues collected from the irrigated compartment compared with samples from the dry compartments. Root tissue samples from the dry/intact compartment had $\delta^2H$ values two times higher than samples from the dry/girdled compartment. These preliminary results show that under drought stress, some water flows from the wet roots to the leaves via the xylem and is then recycled from the leaves to the dry roots via the phloem. A better understanding of phloem function in whole-plant water transport will help to understand the plant mechanisms involved in the irrigation strategy termed partial root-zone drying.

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## Field Testing of an Automated Canopy Temperature-Based Water Stress Index for Precision Irrigation of Winegrape

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In arid winegrape production regions, irrigation precision is limited by the logistical difficulty of monitoring vine water status. In previous research, we developed a model to predict the canopy temperature of a grapevine under well-watered conditions and used predicted and measured canopy temperatures to calculate a water stress index. The objective of this research was to automate data acquisition, calculation, and display of the index and to relate index values with irrigation events, soil moisture, and other indicators of vine water status. A sensor network system that included infrared radiometers, a weather station, soil moisture probe, tipping bucket rain gauge, and data logger was installed in commercial plots of Malbec and Chardonnay at different vineyard sites in southwestern Idaho. Data were acquired remotely in real time and a daily crop water stress index was calculated and displayed graphically on a web-based user interface that was accessible for use by vineyard managers. Daily water stress index values decreased after an irrigation event and increased between irrigation events. The depth of water penetration during an irrigation event differed by vineyard and corresponded with irrigation event duration. Values of midday leaf water potential corresponded with water stress index values; however, the water stress index was more responsive to irrigation events than leaf water potential. Results from the first year of this two-year field trial suggest that this methodology can provide a real-time, automated daily indicator of vine water status for use as a decision-support tool in a precision irrigation system.

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## Enology and Viticulture – CONTINUED

### From Plant Water Status to Wine Flavonoid Composition: A Precision Viticulture Approach in a Sonoma County Vineyard

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Ecophysical variation affecting wine flavonoid composition in a Cabernet Sauvignon/110R vineyard was modeled over two growing seasons. The research site received 39 mm precipitation in 2016 and 162 mm in 2017. Soil properties of the vineyard were proximally sensed to acquire soil texture. An equidistant 30 × 30 m grid was overlaid to characterize grapevine primary and secondary metabolism. The midday stem water potential ($\Psi_{stem}$) integrals were calculated and clustered by k-means into two water status zones: severely stressed (Zone 1) and moderately stressed (Zone 2) that explained 70% of water status variation. The surface soil texture explained 84% of the variation in $\Psi_{stem}$ while subsurface soil texture explained 80%, depending on the loam to sandy loam contribution. The primary metabolism decoupled when Zone 2 reached 26 and 24 Brix in 2016 and 2017, respectively, with significantly higher Brix values of 30 and 27 in Zone 1. Based on this decoupling in Brix, fruit was harvested differentially from two zones in both years and vinified separately. In 2016, total anthocyanidins were higher in Zone 2. Di- and tri-hydroxylated anthocyanidins were more than two-times concentrated in Zone 2. Myricetin-, quercetin-, kaempferol-3-*O*-glucosides and total flavonols were higher in Zone 2. However, there was no difference in anthocyanidins and flavonols in the second season, except in kaempferol-3-*O*-glucoside, which was lower in Zone 2. The results indicated that in 2016, the water stress between the two zones was great enough to alter flavonoid concentration in base wine. However, in 2017, harvest commenced earlier when the two zones started separating in Brix, and wine flavonoid concentration coalesced accordingly. This study provides fundamental knowledge to coalesce vineyard variability by linking soil texture to plant water status with precision viticulture tools and describes its influence on the final wine product's flavonoid profile.

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### Proximal Soil Sensing for Vineyard Management in Crimson Seedless Table Grape

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A Crimson Seedless vineyard was modeled to examine the ecophysical variation and plant water status influence on productivity and berry chemistry over two years. Electrical conductivity (EC) of the soil was proximally sensed with electromagnetic induction. A stratified random sampling method and an equidistant 30 × 30 m grid sampling were used in 2016 and 2017 to ground-truth proximally sensed data. Deep EC was related to variation of plant water status in 2016 ($R^2$ = 0.4897); however, shallow EC did not explain the variation in water status.

## Enology and Viticulture – CONTINUED

Grape primary metabolism was measured for total soluble solids, total acidity, pH, and berry weight. Only pH in 2016 and total acidity in 2017 showed significant differences based on different degrees of water stress. Secondary metabolites were characterized with a C18 reversed-phase HPLC. The results indicated that peonidin was the most dominant anthocyanidin form in Crimson Seedless table grape. There was no difference in anthocyanidins in 2016. However, delphinidin, petunidin, and malvidin were greater in content within the relatively higher water stress cluster. This study provides evidence to optimize the application of proximal sensing to monitor, estimate, and manage on-site measurements of plant water status, productivity, and berry chemistry in a large-scale vineyard.

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### Grapevines Hit with a Double Whammy: Effects of Water Stress during Heat Waves on Growth and Physiology

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Deficit irrigation is a common management tool in viticulture, especially in arid and semiarid regions. However, recurring heat waves in recent years have posed challenges for grapevines under water stress. The combination of heat stress and soil water deficit may lead to undesirable impacts on grapevine growth and grape ripening. To understand the interactive effects of heat and water stress, two contrasting cultivars, Cabernet Sauvignon and Riesling, were chosen for this study. Treatments included water stress only, heat stress only, the combination of water and heat stress, and a no-stress control. Treatments were imposed in climate-controlled growth rooms twice (before and during veraison) and lasted seven days each time. Each treatment round was followed by a seven-day recovery period. In both cultivars, vines under water stress had reduced shoot growth and leaf gas exchange, regardless of the presence or absence of heat. Heat stress alone had less-pronounced adverse effects on vegetative growth than did water stress. Additive negative effects of the combination of stresses were found on leaf water potential and sometimes on vegetative growth. Significant interactions between water and heat stress were found for leaf gas exchange in both cultivars. Contrary to expectations, the relationship between leaf water potential and stomatal conductance was similar in these two cultivars. In terms of berry ripening, the two cultivars differed in their responses: water stress advanced the onset of ripening in Cabernet Sauvignon, but heat stress had no effect; however, the opposite occurred in Riesling. These preliminary results indicate that, compared with heat stress, water stress is the dominant factor affecting grapevine growth and gas exchange. The interplay of heat and water stress on grape ripening is more complicated, and requires further investigation.

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