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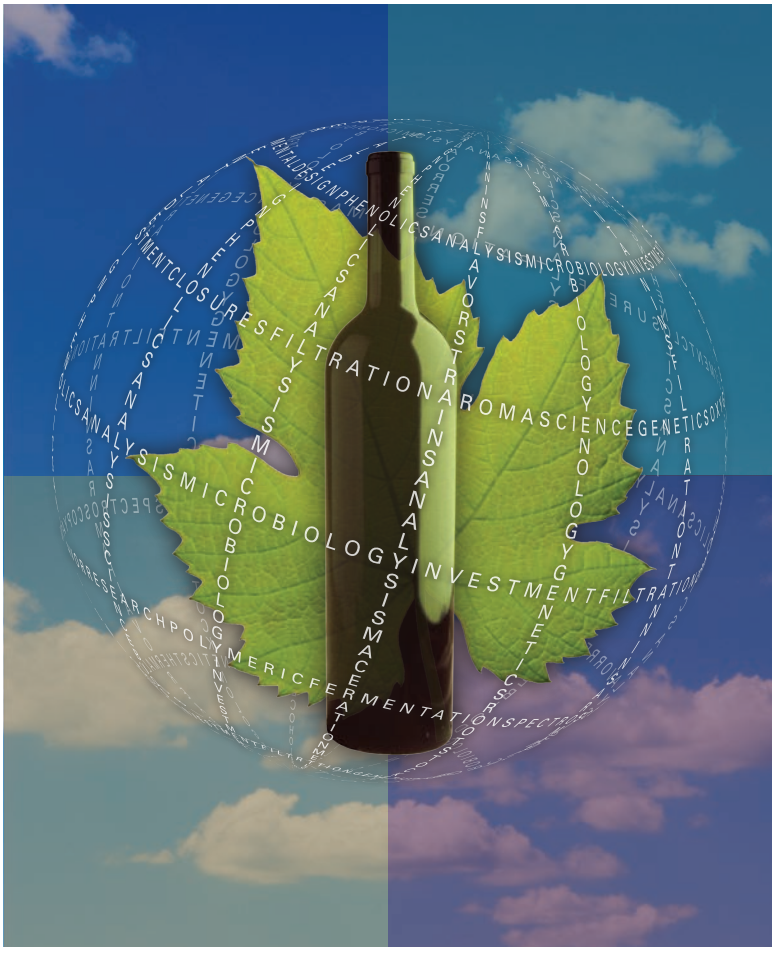

TECHNICAL ABSTRACTS

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2017 National Conference Technical Abstracts

Oral Presentation Abstracts

Wednesday, June 28

Thursday, June 29

Poster Presentation Abstracts

Wednesday, June 28 & Thursday, June 29

NOTE: pp. 1-47 constituted the program section of the original printed *Program and Technical Abstracts* booklet.

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

Alphabetical Listing of All Authors with Submitted Abstracts

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

Enology — Wine Aroma Session

Impact of Intercontinental Bulk Wine Shipping Conditions on the Aroma and Sensory Profile of Chardonnay

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How shipping of bulk wine affects the aroma and sensory profile of wine is attracting increased attention. High temperatures may occur in containers when bulk wine is shipped long distances. Elevated temperatures may adversely affect aroma and color of wines. We compared the record of environmental conditions during intercontinental bulk wine shipping with analysis of the aroma and sensory composition after transportation. Three bulk wine shipments, each including six containers filled with 24,000 L Chardonnay in flexitanks or ISO-tanks, were investigated. The intercontinental shipping route was scheduled for different seasons over one year. For reference, samples of the identical wine were dispatched via air freight express. The six containers were either placed at three different positions on the vessel or equipped with different isolation materials to find out how to optimize the shipping process. Wine temperatures reached 36°C in a worst-case scenario. Different positions on the vessel made an average difference of 7°C. Isolation could lower the maximum temperature by 10°C. Wine at the tank surface responded much faster to the outside air temperature, resulting in temporary gradients of 12°C during dwell time periods in marine terminals with heavy sun radiation and no movement of the container. Temperature recordings were segmented into 5°C steps and multiplied with exposure time. Time-temperature integrals were correlated with descriptive sensory and GC × GC data. PLS analysis revealed that pineapple, banana, and citrus flavor decreased significantly when wines were exposed to temperatures >25°C for >60 days, >30°C for >10 days, or >35°C for <1 day. A significant decrease in ethyl and acetate esters occurred even earlier under the same temperature conditions.

Funding Support: German Ministry of Economics, Vineris Winery, Lidl

Characterizing Glycosidically-Bound Sensory Precursors in Smoke-Exposed *Vitis vinifera* Berries

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Volatile phenolic compounds like guaiacol and their glycosides influence the sensory attributes of wine made from smoke-exposed *Vitis vinifera* berries and correlate with unpleasant "smoky," 'ashy," "burnt meat," and "Band-Aid" aromas. Understanding this phenomenon is paramount because much of the North American winegrape crop is produced near forest fire-prone regions. To date there has been a lack of detailed characterization of the phenolic glycosides, with the majority putatively assigned in the absence of direct empirical evidence. However, phenolic glycosides constitute a "sensory potential" that can negatively impact the sensory profile of a wine following fermentation or after bottling. This hinders efforts to develop remedial and preventative strategies, precludes absolute quantitative assessment of the total pool of volatile phenolic compounds, and confounds any correlation between volatile phenolics and their potential impact on wines made

from smoke-exposed berries. The goal of this study was to develop a comprehensive workflow to characterize phenolic glycosides, including their glycosidic linkages and anomeric chemistry, and to facilitate potential identification of acidic or modified glycones. Guaiacyl and syringyl glycosides were synthesized and used as model systems to develop analytical methods, as these putative glycosides represent the best (albeit incomplete) report of the observed glycosides present in smoke-exposed berries. A combination of exoglycosidase enzymes, uHPLC-MS, GC-MS, and CE-MS was used to develop and validate the analytical workflows. Method validation results for quantification of phenolic glycosides in smoke-exposed berries are shown. The developed analytical strategies can be extended to other glycosylated metabolites integral to wine quality like terpenoids or norisoprenoids, which are predominantly biosynthesized as glycosidically-linked precursors.

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Myths and Facts about the Role of Precursors in the Formation of "Reductive Aromas" in Wines Postbottling

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Volatile sulfur compounds (VSCs) are important wine compounds that impart "reductive" aromas that negatively impact wine aroma and quality. Hydrogen sulfide (H_2S) and methanethiol (MeSH) are two of the main compounds associated with "reductive" aromas in wines postbottling. Precursors previously suggested as playing important roles in modulating VSC concentrations in wines postbottling were evaluated. The ability of sulfur-containing amino acids such as cysteine (Cys), methionine (Met), and glutathione (GSH) and other low-molecular-mass sulfur compounds to act as precursors to H_2S and MeSH were assessed in real wines. Cys and GSH did not act as precursors to H_2S on a wine-relevant scale. The presence of copper remained the main factor that contributed to significant increases in H_2S concentrations from yet-to-be-identified precursor compounds. Cys and GSH were associated with reduced H_2S formation from copper-catalyzed reactions in Shiraz wines, either by inhibiting H_2S formation from copper-catalyzed reactions or possibly by forming mixed disulfides through the reaction of Cys/GSH with H_2S. Only dimethyldisulfide (DMDS) and methylthioacetate (MeSAc) contributed significantly and on a wine-relevant scale to MeSH concentrations in wines postbottling. In all instances, the presence of copper had significant effects on the ability of precursors to produce H_2S and MeSH and on the rate of H_2S and MeSH formation. Wine pH also significantly affected MeSH formation. This study demonstrated that Cys, Met, and GSH do not pose a risk of H_2S or MeSH formation in wines postbottling. However, the presence of MeSAc, or a disulfide such as DMDS, posed a significant risk of MeSH formation, with up to a 20% MeSH yield and a 70% MeSH yield obtained from MeSAc and DMDS, respectively, in wines over a twelve-month storage period.

Funding Support: Australia's grapegrowers and winemakers through their investment body, Wine Australia, with matching funds from the Australian Government

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Enology — Wine Aroma Session – CONTINUED

Use of Glutathione in White Winemaking – Friend or Foe?

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Recently two OIV resolutions (OENO 445-2015 and OENO 446-2015) were adopted, defining the use of glutathione (GSH) up to a maximum concentration of 20 mg/L in must and wine. Studies have shown the benefits of GSH addition, especially in Sauvignon blanc wines. We evaluated the impact of added GSH on oxidation product formation in model wines, which was compared to ascorbic acid and sulfur dioxide additions. The analysis of oxidation products formation focused on determining yellowish-colored xanthylium compounds by LC-ESI-ToFMS and acetaldehyde by HS-GC-FID. The results showed that, under certain conditions, GSH did not inhibit carboxymethine-bridged (+)-catechine dimer formation and subsequent xanthylium cation pigment generation, unlike ascorbic acid or sulfur dioxide, both of which provided a good protection against oxidative color changes. In systems containing 0.08 to 0.32 mmol/L GSH without any further addition of SO_2 or ascorbic acid, increased acetaldehyde concentrations were observed. Next, we investigated the effect of GSH addition to white wine with respect to color development, sensory expression, and sulfide off-flavor formation. Riesling, Sauvignon blanc, and Chardonnay grapes were processed under different conditions and musts were obtained with different phenolic concentrations. The addition of GSH as a pure substance or the use of GSH-rich inactivated yeast preparations (IDY), either prior to or after fermentation, resulted in wines with varying GSH concentrations. GSH treatment yielded lighter-colored musts, but there were no differences in the yellow color of the bottled wines. GSH and IDY addition prior to fermentation increased 3-mercaptohexanol concentrations in Sauvignon blanc wines. Excess GSH treatment of musts and wines before bottling led to increased formation of off-flavored sulfur volatiles (e.g., H_2S, dimethyl sulfide), determined using a novel HS-SPME-GC method followed by SIDA quantification, negatively influencing the wines' sensory quality.

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Enology — Wine Aroma Session – CONTINUED

Free Monoterpene Isomer Profiles of Eight *Vitis vinifera* L. cv. Varietal White Wines

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Monterpene compounds are important to varietal white wines, but the isomer profiles of these compounds are less well known. This study evaluated the monoterpene isomer profiles and enantiomeric percentages of white wines from diverse grape varieties. One hundred forty-eight wines, including Chardonnay, Gewürztraminer, Muscat, Pinot gris, Riesling, Sauvignon blanc, Torrontes, and Viognier, were collected from 2012 and 2013 vintages. Seventeen monoterpene isomers: *S*-(-)-limonene, *R*-(+)-limonene, (2*R*,4*S*)-(+)-*cis*-rose oxide, (2*S*,4*R*)-(-)-*cis*-rose oxide, (2*R*,4*R*)-(-)-*trans*-rose oxide, (2*S*,4*S*)-(+)-*trans*-rose oxide, (2*R*,5*R*)-(+)-*trans*-linalool oxide, (2*R*,5*S*)-(-)-*cis*-linalool oxide, (2*S*,5*S*)-(-)-*trans*-linalool oxide, (2*S*,5*R*)-(+)-*cis*-linalool oxide, *S*-(-)-nerol oxide, *R*-(+)-nerol oxide, *R*-(-)-linalool, *S*-(+)-linalool, *S*-(-)-α-terpineol, *R*-(+)-α-terpineol, and *R*-(+)-β-citronellol were identified and quantified by headspace-SPME-MDGC-MS. Linalool oxide isomers characterized Chardonnay, Pinot gris, and Sauvignon blanc wines. α-Terpineol, linalool, and linalool oxide isomers were abundant in Gewürztraminer, Muscat, Torrontes, and Viognier wines. Linalool oxide and α-terpineol showed higher concentrations in Riesling wines. Interestingly, the isomers of each compound had very similar profiles in the same varietal wine. Separation between varieties was achieved using discriminant analysis in spite of high variability among vintage, region, and wine style. The difference in monoterpene isomer profile and enantiomeric percentages in varietal wines may contribute to the sensory differences among these wines.

Funding Support: Oregon Wine Board, Oregon Wine Research Institute

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Viticulture — Water Relations Session

Changes in Grape (*Vitis vinifera* L.) Berry Cuticle during Fruit Development in Response to Water Deficit Stress

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In grapes, the cuticle protects the berry from water loss, which may in turn affect the final berry size at ripening. Water deficit (WD) is a common stress in vineyards, but little is known about how the berry cuticle is modified in response to this stress. Our previous work showed that the expression of some cuticle genes is modulated in grape berries exposed to water deficit stress. Furthermore, differential expression and gene regulatory network analyses of grapevine RNA-seq datasets indicated that the cuticular wax biosynthetic pathway is differentially regulated during berry development and in response to environmental stresses, including water deficit. Thus, we hypothesize that in response to WD stress, the developing grape berry will modify the composition of cuticular waxes on the berry surface to reduce the rate of water loss. To study this question, we carried out experiments in 2015 and 2016 to characterize changes in transpiration and cuticular wax composition of developing Merlot berries in response to WD stress and found that the rate of water transpiration did not change in response to WD stress. Nevertheless, we expect changes in cuticular wax composition and in expression of cuticular wax biosynthetic genes in response to WD stress, since our experiment in 2015 indicated changes in berry wax composition. We are currently analyzing the wax composition and expression of key cuticular genes in the samples collected in 2016 to confirm our results.

Funding Support: NSERC Discovery Grant

Berry-Splitting Resistance of Merlot, Syrah, Zinfandel, and Concord

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Grape berry-splitting results from cuticle failure due to excessive tensile stress on the skin. Split berries expose flesh cells to the atmosphere. Without the protection of the hydrophobic cuticle, splitting leads to dehydration and increases susceptibility to pathogen infection. If the resistance to splitting could be measured, varietal differences could be better understood. The objectives of this research were: (1) to determine varietal differences, and (2) to understand developmental changes in resistance. Based on a thin shell theory, we simplified splitting resistance as the maximum internal pressure at the time a berry splits. This study further investigated the resistance of Merlot, Syrah, Zinfandel, and Concord. Berry clusters were collected, and berries were categorized by total soluble solids. Five berries from each cluster were sampled and mounted on injection needles. An apparatus was designed to inject liquid into berries and to accumulate internal pressure. The hydraulic pressure in the berry and berry expansion were recorded

automatically by a data logger. Cuticle thickness was examined by confocal microscopy. The resistance to splitting decreased greatly after the onset of berry softening in all varieties, suggesting that berry splitting is unlikely to be observed before softening. Among the four varieties, Merlot had the highest resistance to internal pressure. Correlation analysis against soluble solids confirmed that Merlot is more splitting-resistant throughout ripening than the other tested varieties. Merlot and Zinfandel had similar cuticle thickness, thus cuticle thickness was insufficient to explain the superior resistance of Merlot. Moreover, Concord berries had a thicker cuticle than, but similar resistance to, Zinfandel and Syrah berries.

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Estimates of In Situ Hydraulic Conductance between the Stem and the Berry in *Vitis vinifera* Chardonnay

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The hydraulic connection of the grape (*Vitis vinifera* Chardonnay) berry and its parent vine changes over berry development between pre- and postveraison and is a critical element for understanding the response of berry size and expansive growth to changes in vine water status. The hydraulic conductance pathway has been estimated using excised material, but estimates vary greatly and span over one order of magnitude. Here we used a nondestructive approach, applying pressure steps to the root system of a non-transpiring vine in dry soil to manipulate shoot water status, while simultaneously measuring the change in stem water potential of the shoot and the change in berry diameter. Postveraison hydraulic conductance was ~10% of preveraison conductance, consistent with previous research showing hydraulic conductance decreasing over berry development. The estimated pre- and postveraison conductance was similar to the lower conductance values previously reported and indicated that low hydraulic conductance postveraison could minimize water backflow from the berry to the parent vine. This study provides the first in situ estimate of hydraulic conductance between the berry and the stem, and the method should be applicable to other plants to study water relations between fruit and parent plant.

Funding Support: California Table Grape Commission, UC Davis Department of Plant Sciences, UC Davis Graduate Group in Horticulture & Agronomy, UC Davis Office of Graduate Studies, China Scholarship Council, Curtis J. Alley Memorial Research Scholarship, Robert L. Balzer Scholarship, Horace O. Lanza Scholarship, Wine Spectator Scholarship, and Henry A. Jastro Graduate Research Award

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Viticulture — Water Relations Session – CONTINUED

Hydraulic and Chemical Signals May Unravel the Basis of Isohydry in Grapevines Subjected to Water Stress and Recovery

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Cultivar-specific differences in stomatal responses to soil moisture deficits, known as isohydry, are well documented in grapevine; however, the basis for these differences is less well understood. In 2016, we conducted a study to investigate the basis of isohydry using potted *Vitis vinifera* Shiraz and Grenache on a Drought-Spotter automated gravimetric platform. This platform allowed precise control of soil moisture and quantification of the daily dynamics of canopy transpiration of individual potted vines. Leaf and stem water potentials, leaf stomatal conductance (g_s), root hydraulic conductance (L_o), and leaf hydraulic conductance (K_{leaf}) were measured at several timepoints over the course of soil dry-down and rehydration. At the same timepoints, xylem sap and leaf and root samples were collected to determine abscisic acid (ABA) concentrations and aquaporin (AQP) gene expression levels, respectively. We hypothesized that the putatively more isohydric Grenache regulates its stomata under water stress by decreasing root and/or leaf hydraulic conductance, while the relatively anisohydric Shiraz maintains L_0 and K_{leaf} under water stress. Withholding irrigation lowered g_s to near stomatal closure in both cultivars within three days. Well-watered Grenache vines increased L_0, possibly in response to high g_s; this response was not observed in water-stressed vines. In contrast, L_0 tracked g_s closely in both well-watered and water-stressed Shiraz vines, indicating a high degree of hydraulic coupling between roots and shoots in this cultivar. Under water stress, AQP gene expression levels decreased in Grenache leaves but increased in roots, while water stress increased both leaf and root AQPs in Shiraz. Xylem ABA concentrations were higher in Grenache, which could explain the downregulation of specific leaf AQPs. These findings suggest that Grenache may be relatively isohydric due to regulation of both hydraulic and chemical signals while Shiraz is relatively anisohydric due predominantly to hydraulic regulation.

Funding Support: Australian Research Council Centre of Excellence in Plant Energy Biology

Viticulture — Water Relations Session – CONTINUED

Grapes and Irrigation: Of Myths and Dogmas

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Irrigation is a commonly used management tool to manipulate yield and quality of grapes, especially in arid and semiarid regions. However, long-standing myths still have strong influences on vineyard irrigation practice. A better understanding of water relations of grape berries is fundamental to applying evidence-based irrigation practices. Contrary to previous beliefs, by tracing water movement in the xylem with xylem-mobile dye, it was found that berries remain hydraulically connected to the mother vine. When the pressure in the xylem was manipulated, inward dye movement into ripening berries (xylem inflow), which normally ceases at veraison, was restored and outward dye movement from berries (xylem backflow) was stopped. Using a fruit growth model, it was estimated that, to meet the demand of berry sugar accumulation, phloem inflow exceeded the water demand for berry growth and transpiration. Along with apoplastically unloaded sugar, this surplus phloem water may alter the pressure gradient inside the xylem and thus the direction of xylem flow during berry ripening. As a practical implication, the surplus phloem water buffers ripening berries from changes in xylem water supply; therefore, unlike unripe berries, ripening berries become insensitive to xylem water supply (e.g., water taken up by roots from the soil). Consequently, the most effective time to control berry size is before ripening, and postveraison drip irrigation does not enlarge berries by adding water to them. It is thus recommended to apply adequate irrigation after veraison to maintain a healthy canopy for sugar accumulation in the berries and to replenish storage reserves in the permanent structures. Avoiding excess water stress close to harvest may also alleviate berry weight loss (dehydration) during extended hang-time.

Funding Support: USDA Northwest Center for Small Fruits Research, Washington State University, Chateau Ste. Michelle Distinguished Professorship, and Rhone Rangers

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Enology — *Brettanomyces* Management Session

Impact of *Oenococcus oeni* on *Brettanomyces bruxellensis* Growth in Wine

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Wine is particularly vulnerable to *Brettanomyces* infection during and shortly after malolactic fermentation (MLF), as SO_2 cannot be added until this process is complete. Conducting a rapid MLF initiated by inoculation of a commercial *Oenococcus oeni* strain is a useful strategy to prevent *Brettanomyces* spoilage, as this minimizes the length of time the wine is not protected by SO_2. Previous research in our lab demonstrated that *B. bruxellensis* growth was hindered by the presence of live *O. oeni* cells present post-MLF. The current study investigated how long this inhibition persisted post-MLF. Pinot noir wine was inoculated for MLF with three commercial *O. oeni* strains and *B. bruxellensis* was inoculated into the wine 0, 30, and 100 days after completion of MLF. *O. oeni* populations in the wines were still high directly after completion of MLF, and *B. bruxellensis* populations declined rapidly after inoculation. Thirty days post-MLF, high populations of two *O. oeni* strains were still present in the wine but no culturable cells were detected in the wine for the third strain. When *B. bruxellensis* was inoculated into the wine containing no culturable *O. oeni* cells, it grew well. In contrast, *B. bruxellensis* populations declined rapidly when inoculated into wine where there were still culturable *O. oeni* cells. One hundred days post-MLF, no culturable *O. oeni* cells were detected in any wines and *B. bruxellensis* grew well in all but one wine. These findings suggest that MLF may offer limited protection against *B. bruxellensis* infection due to the presence of live *O. oeni* cells. Additional work is being conducted to determine whether other *B. bruxellensis* strains are also sensitive to the presence of live *O. oeni* cells.

Funding Support: Northwest Center for Small Fruits Research

Reduction of *Brettanomyces bruxellensis* Populations from Oak Barrel Staves Using Steam

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Brettanomyces bruxellensis is a spoilage yeast associated with oak barrels used for aging of wines. Infected barrels made from French (*Quercus petraea*) or American (*Q. alba*) oak were used to determine the impact of different toasting levels (low or high), size (16 or 225 L), or age (one or three years) on yeast populations and penetration into staves. In addition, time/temperature combinations using steam were explored as means to reduce, if not eliminate, resident populations. Penetration of the yeast into states was evaluated by collecting shavings prepared by a Forstner drill bit which were then incubated in a *Brettanomyces* recovery medium. Additional staves were cut into 3 × 10 cm blocks and randomly assigned to steam treatments lasting 0 to 12 min. Following heating, blocks were sawn into 4 mm thick layers and assessed for yeast viability by placement in a nutrient-rich red wine for >60 days. Yeast penetration studies revealed populations of 10^3 cfu/mm^3

in the layer closest to the wine (<2 mm) with lower values (≤10^2 cfu/mm^3) at 2 to 4 mm deep. French oak staves consistently contained higher populations than American oak and penetrated farther (up to 8 mm) regardless of barrel size or age ($p < 0.05$). If located at a depth of ≤4 mm, exposure to steam for a minimum of 9 min was required to no longer recover viable yeasts. A minimum heating of 12 min was necessary to achieve the same extent of reduction for yeast penetration of 5 to 9 mm. Different strains of *B. bruxellensis* yielded similar temperature/time reductions ($p \geq 0.05$). When present within 9 mm of the inner surface, a steaming time at least 12 min is recommended to effectively reduce *B. bruxellensis* in infected oak barrels.

Funding Support: Washington Wine Advisory Committee, Ivory Tower Scholarship Fund

Inactivating *Brettanomyces bruxellensis* in Different Table Wines Using High-Pressure Processing

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Brettanomyces bruxellensis is a major spoilage concern for the wine industry worldwide, leading to undesirable sensory properties and economic losses. Sulfur dioxide is currently the preferred method for wine preservation. However, due to its negative effects on consumers, new, alternative, non-thermal technologies are increasingly being investigated. Therefore, in this study, high-pressure processing (HPP) technology was used to treat different types of table wines. The effects of HPP pressure, yeast strain, alcohol content and pH on inactivation of *B. bruxellensis* were also determined. Additionally, the inactivation kinetics were modelled. Processing at 200 MPa for 3 min resulted in 5.8 log reductions. To achieve high throughput in the wine industry, HPP of 400 MPa for 5 sec (>6 log reduction) is recommended to inactivate *B. bruxellensis*. It was also found that yeast strain influenced HPP inactivation, with AWRI 1499 being the most resistant strain among three investigated. Wine type affected *B. bruxellensis* inactivation, with 200 MPa for 60 sec leading to log reductions ranging from 1.12 ± 0.01 (Dolcetto Syrah) to 5.05 ± 0.07 (Rosé). Alcohol concentration above 12.0% v/v had a significant effect on *Brettanomyces* inactivation increasing inactivation from 3.05 ± 0.08 (12% v/v) to 4.23 ± 0.05 (14% v/v) after 200 MPa for 180 sec. The Weibull model successfully described the non-linear HPP inactivation of *Brettanomyces* in different wines. HPP is a viable alternative for inactivation of *B. bruxellensis* in wine, with the potential to reduce the industry's reliance on sulfur dioxide.

Funding Support: University of Auckland

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Viticulture — Canopy Management Session

Partial Solar Radiation Exclusion with Colored Shade Cloths May Improve Red Winegrape Composition

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The incidence of solar radiation on red grapes can promote synthesis of phenolic compounds desirable in wine production. However, excess solar radiation may induce oxidative damage and increase berry temperature. Under such conditions, metabolites such as organic acids, flavonols, proanthocyanidins, and anthocyanins may break down, leading to worsened quality. We performed a shade cloth trial to evaluate whether these can mitigate the deleterious effect of overexposure to solar radiation. The experiment was conducted in Oakville, CA, on Cabernet Sauvignon with a relaxed VSP in rows oriented NW to SE. The fruit zone was covered with four polyethylene 1-m curtains (20% shade Pearl, 40% shade Blue, 40% shade Red, 40% shade Black, and 40% shade Aluminet) with different optical properties placed at the fruit zone and a control with no cover. Grape berries were sampled at six developmental stages to determine berry mass, total soluble solids (TSS), pH, total acidity (TA), total flavonols, anthocyanins, and proanthocyanidins. Berry temperature was 3.7°C higher on the west side of the control than on Black. Berry weight under Aluminet was lower than the other treatments. There was a non-significant trend in TSS where Pearl, Aluminet, and control had higher values than Blue and Black. Berries under the cloths had often significantly lower pH and higher TA than control, but only Black showed significant effects at harvest. Berry anthocyanin content tended to be higher under the nets, while flavonol content was significantly higher in the control. Proanthocyanidin content at harvest was higher in Black. Mean degree of polymerization (mDP) of proanthocyanidins was not affected by treatments. Our results provided evidence that shade cloths may mitigate temperature spikes while transmitting enough radiation into the fruit zone to achieve a better grape composition than uncovered grapes.

Funding Support: HP Olmo Research Funds

Efficiency of Leaf Removal and Cluster Thinning Are Modulated by Climatic Conditions in Cool Climate Viticulture

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One of the most important challenges of cool climate viticulture is to achieve technological fruit maturity consistently each year. Several viticultural practices are usually implemented in vineyards to advance fruit maturity and maximize berry quality. Cluster thinning is commonly used in premium grapegrowing regions to alter the sink/source ratio, advance fruit ripening, and improve fruit primary metabolism. Leaf removal in the cluster zone is used to expose the clusters to sunlight and improve canopy microclimate, reduce disease pressure, and promote secondary metabolism. We investigated the combination of cluster thinning and leaf removal before veraison on Cabernet franc vines grown in a cool climate over

two seasons (2011 and 2012). Fruit ripening advanced quickly once cluster thinning or leaf removal was implemented. However, the dynamics of fruit ripening from veraison to harvest were very distinguishable between the two very different growing seasons. 2011 was a cooler year with lower ambient temperatures and low solar radiation than 2012. Leaf removal and cluster thinning significantly improved anthocyanin accumulation between 10 and 20 days after veraison in 2011. However, the boost of anthocyanin accumulation was not significant in 2012. Anthocyanin accumulation efficiency, indexed as the anthocyanin:sugar ratio, increased more dramatically than the control in 2011 than in 2012. In addition to berry ripening, leaf removal and cluster thinning also led to a more uniform berry composition at harvest than the control. However, in the warm year (2012), this effect was not significant. The effects of leaf removal and cluster thinning on berry ripening, especially on anthocyanins, were highly modulated by the seasons, and treatments may be more beneficial for fruit ripening and berry composition at harvest when the season is cooler than an average vintage.

Funding Support: AgBioResearch at Michigan State University (Project GREEEN); Michigan Grape and Wine Industry Council; MSU Southwest Michigan Research and Extension Center

Proanthocyanidin Compositional Shifts Are Modulated by Canopy Gaps and Applied Water in Winegrape

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The relationships between variations in grapevine (*Vitis vinifera* L. cv. Merlot), fruit zone light exposure, and water deficits and the resulting berry flavonoid composition were investigated in a hot climate. The experimental design involved application of mechanical leaf removal (control, prebloom, post-fruit set) and differing water deficits (sustained deficit irrigation and regulated deficit irrigation). Flavonol and anthocyanin concentration increased with prebloom leaf removal in 2013, but with post-fruit set leaf removal in 2014. Post-fruit set leaf removal increased total proanthocyanidin concentration in both years, while no effect of applied water amounts was observed. Mean degree of polymerization of skin proanthocyanidins increased with post-fruit set leaf removal compared to prebloom, while water deficit had no effect. Conversion yield was greater with post-fruit set leaf removal. Seed proanthocyanidin concentration was rarely affected by applied treatments. The application of post-fruit set leaf removal, regardless of water deficit, increased the proportion of proanthocyanidins derived from the skin, while no leaf removal or prebloom leaf removal, regardless of water deficits, increased the proportion of seed-derived proanthocyanidins. The study provides fundamental information to viticulturists and winemakers on how to manage red wine grape low molecular weight phenolics and polymeric proanthocyanidin composition in a hot climate.

Funding Support: American Vineyard Foundation

Enology — Molecular Microbiology Session

Role of the Microbial Community in Problematic Fermentations

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Instances of problematic fermentations arise periodically during wine production in spite of careful fermentation management practices. The initial microbial load of grape must is one factor known to impact yeast fermentation. We are focused on understanding the mechanism underlying yeast fermentation performance in the presence of lactic and acetic acid bacteria in the grape must. Although only some *Lactobacillus* species, including *Lactobacillus kunkeei,* are associated with fermentation arrest, both lactic and acetic acid bacteria have been isolated from problematic fermentations. Our results show that many of these bacteria can induce the [*GAR*$^+$] prion in yeast. The [*GAR*$^+$] prion alters yeast metabolism, reducing fermentation rate and decreasing the ability of the yeast to dominate the fermentation. Acetic acid, a common bacterial metabolite, was found to induce the [*GAR*$^+$] prion. However, in commercial samples the simultaneous presence of acetic acid bacteria and *L. kunkeei* is often observed, suggesting that acetic acid production alone is not the sole factor leading to arrest. Therefore a metabolomics analysis was performed, comparing the spent media of inducing and non-inducing acetic acid bacteria. Higher levels of caprylic acid and oxidized glucose intermediates were found in the medium of the inducing bacteria. None of these compounds induced the prion state in yeast. Acetic acid levels did not vary across inducing and non-inducing strains of bacteria. The presence of glucose derivatives known to bind to SO_2 may explain the fermentation problems that arise even when SO_2 is added and SO_2-sensitive inhibitory bacteria are also present. Thus, the microbial community plays an important role in enabling inhibition of yeast fermentation by specific community members.

Funding Support: American Vineyard Foundation

Evolutionary Engineering and Breeding of *Saccharomyces cerevisiae* to Increase Acidity and Decrease Ethanol Levels in Wines

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Over the past decades, global warming and its effect on grape ripening, combined with evolution of consumer preferences toward low-alcohol, but full bodied and well-structured, wines has raised a major concern for the wine industry. Producing wines with lower alcohol content became one of the highest challenges. However the conversion rate of sugars into ethanol is very stable among *Saccharomyces cerevisiae* strains. To overcome those physiological limitations, we implemented a combination of evolutionary engineering approaches and classical breeding. Our approach resulted in a strain with decreased ethanol production linked to enhanced production of glycerol but also an increase of acidity, conferring more balance and freshness to wines. Metabolomic, transcriptomic, and genetic studies showed that these modifications are not due to deregulation or mutation of genes

directly involved in the glycerol synthesis pathway, but to major changes in carbon, energy, and redox metabolism that are probably under multigenic control. To identify the molecular basis of these new phenotypes, QTL mapping was implemented using bulk segregant analysis (BSA) and combined with whole-genome sequencing of the evolved strains. Regions of the genome linked to the phenotypes have been highlighted and candidate genes are under functional validation.

Funding Support: Institut National de la Recherche Agronomique

Molecular Characterization of *Saccharomyces cerevisiae* during Fed-Batch Fermentation of High-Gravity Grape Musts

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High-gravity grape musts are the basis of premium and sweet wines. Their sugar content can reach up to 400 g/L. Those high sugar amounts may increase osmotic stress for the wine yeast *Saccharomyces cerevisiae* and increase amounts of undesired secondary metabolites such as acetic acid and acetaldehyde, which may damage the quality of the final product. We investigated the metabolic and transcriptional response of *S. cerevisiae* to the high sugar content of high-gravity grape musts. The stress response is divided into an initial stress adaption to the high-sugar environment, followed by biosynthesis and release of several secondary metabolites from the glycolytic pathway. To reduce the stressful conditions for the wine yeast and the biosynthesis of undesired byproducts, we applied fed-batch technology to ferment the high-gravity grape musts. We investigated the synthesis and release of the most important fermentation byproducts and the transcriptional response of the wine yeast during fed-batch fermentations in comparison to regular batch fermentations. Under fed-batch conditions, the *S. cerevisiae* can ferment greater amounts of sugar and releases smaller amounts of problematic secondary metabolites such as acetic acid and acetaldehyde. We used gene expression analyses to investigate expression of marker genes related to osmotic stress response, such as stress-inducible acetic acid biosynthesis. In addition, we monitored the regulation of key genes in certain parts of the glycolytic pathway during the fermentation process. Our data show good correlation of transcriptional and metabolic activity and give insight into the molecular processes during fed-batch and batch fermentation processes of high-gravity musts.

Funding Support: FHproUnt (lead partner Jülich), Federal Ministry of Education and Research (Federal Republic of Germany), Federal Institute of Viticulture and Horticulture Neustadt, Germany (DLR Rheinpfalz, Neustadt), University of Applied Sciences Kaiserslautern, Germany, University Geisenheim

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Viticulture — General Viticulture Session

Causal Role of *Drosophila* spp. in Sour Rot Development

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Sour rot is a disease complex characterized by rotting of the grape berry partnered with internal development of acetic acid and is typically associated with the abundant presence of *Drosophila* fruit flies in the vineyard. Acetic acid production within rotted berries is a two-step process, requiring yeasts first to produce ethanol and bacteria subsequently to oxidize this to acetic acid, and we have characterized the microorganism species involved. To investigate potential microbial and nonmicrobial contributions of *Drosophila* spp. within the sour rot complex, we produced axenic *D. melanogaster* eggs using standard protocols and reared larvae under axenic conditions, yielding adults devoid of gut or surface microbiota, and compared their effects with wild-type flies. Wounded berries exposed to wild-type *D. melanogaster* for eight days developed sour rot symptoms, but berries in the presence of axenic flies required a co-inoculation with *Saccharomyces cerevisiae* and *Gluconobacter oxydans,* by dipping the wounded berries into an aqueous suspension of the two organisms, for sour rot to develop. In contrast, wounded berries dipped in the aqueous suspension but unexposed to flies did not develop sour rot symptoms, nor did wounded berries exposed to axenic flies but not inoculated with microorganisms. In related experiments to compare individual *Drosophila* species, we saw similar contributions from *D. suzukii* (Spotted Wing Drosophila) and *D. melanogaster.* Thus, sour rot development appears to require not only the presence of a wounded berry, yeast and acetic acid bacteria, but also *Drosophila* spp. Although wild-type flies in the vineyard clearly can vector microbes that cause the disease, our work indicates that the flies also play a crucial role via some nonmicrobial mechanism. In multiple replicated field trials, antimicrobial plus insecticide sprays consistently provided significant sour rot control, while antimicrobial sprays alone did not.

Funding Support: Specialty Crop Block Grant Initiative, New York State Department of Agriculture & Markets, New York Wine and Grape Foundation

Cold-Climate Cultivar Fall-Acclimation and Fruit-Ripening Alterations in Response to Changing Temperatures

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The lack of mediating water bodies in the continental United States allows for more rapid climactic shifts and subsequent year-to-year unpredictability. The effects of climate change include increased frequency of climactic extremes, increasing effects on the physiological ecology of vines. Moving forward, efforts to breed or identify suitable cultivars based on favorable reactions in response to unpredictable climactic shifts are necessary. An experiment was conducted to compare the effects of temperature on the progression of morphological changes related to fall acclimation response and fruit ripening in three commonly-grown cultivars with differing levels of perceived regional predictability (Frontenac Gris, regionally adapted; St. Croix and Marquette, regionally unpredictable). Twelve indicators relating to growth cessation, tissue maturation, berry growth, and fruit quality were

monitored as photoperiod decreased from 14.5 to 12.5 hr of daylight in five environments. Data reduction was used to extract latent processes from high-level interactions between temperature and morphological trait data in each environment. Correlation was used to identify commonalities among temperature deviations and plant reactions among environments. St. Croix was highly reactive to temperature decreases as with symptomology of water restriction to stem tissue and fruit. Alternatively, Marquette was highly reactive to temperature increases and easily reverted back to active growth, as indicated by increased water allocation to all sink tissues. Frontenac Gris had a moderated response, not as reactive to temperature increase as Marquette, but similar as temperatures cooled. The defining characteristic of Frontenac Gris leading to its relative success in North Dakota likely stems from its unrelenting progress in bud maturation under varied environmental conditions. Results suggest that breeding for favorable temperature reactionary types in addition to early acclimation induction may improve the year-to-year stability in production of future cultivars.

Funding Support: North Dakota Specialty Block Grant North Dakota Grape and Wine Research Grant

A Preliminary Study of Taxonomic and Geographic Relationships among Accessions of *Vitis berlandieri* and Associated Taxa

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Endemic to the limestone soils in central Texas, *Vitis berlandieri* (currently *V. cinerea* var. *helleri*) was incorporated into rootstock breeding programs after scions grafted to early rootstock cultivars exhibited lime-induced iron chlorosis in calcareous soils. Despite its historical and viticultural significance, very few *V. berlandieri* accessions are conserved in germplasm repositories, and information regarding the genetic and phenotypic diversity in the species is sparse. In 2015 and 2016, we collected *V. berlandieri* from central Texas and sampled *V. cinerea* from east Texas. Our objectives were three-fold: (1) expand *ex situ V. berlandieri* germplasm; (2) characterize *V. berlandieri* genetically and geographically, particularly in relation to *V. cinerea*; and (3) examine phenotypic diversity in *V. berlandieri*. Here, we report an analysis of the population structure of *V. berlandieri* and associated species collected throughout Texas and northern Mexico. The analysis included accessions obtained from several germplasm repositories and new accessions. To reduce sampling bias, we included samples of *V. mustangensis*, which is found across the range of *V. berlandieri* and *V. cinerea* within the collection areas, but is morphologically, phenologically, and genetically distinct from both species. Our initial results show strong support for population structure within the sampled accessions and indicate a distinct *V. berlandieri* subpopulation in south-central Texas and *V. cinerea* subpopulations in eastern Texas and northern Mexico.

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Enology — Phenolics Session

Impact of Postveraison Berry Exposure on the Skin Tannin Activity of Partial Extracts

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Previous work conducted on tannin maturation in Cabernet Sauvignon during the 2015 vintage suggested that pigmented tannin concentrations in partial extractions during this period influenced the thermodynamics of tannin interaction with a hydrophobic surface (tannin activity). Based upon these preliminary results, a shade cloth trial was implemented in 2016 to manipulate a variable tentatively identified to have a role in controlling tannin activity during maturation. Treatments were applied at veraison and consisted of 40% and 80% reduction in exposure relative to an ambient control. Quantum sensors were placed within the rows of each treatment to measure photon flux throughout fruit ripening and predawn leaf water potential was taken weekly to assure minimal variation in vine water status among replicates. Berries were sampled every two weeks, starting at veraison, and the skin tissue was separated from pulp and seed by hand, followed by extraction into 16% v/v ethanol for 72 hr. Extracts were analyzed for tannin concentration, average molecular mass, pigmentation, subunit composition, and activity. As in 2015, tannin activity and molecular size distribution declined from veraison until the berries were commercially ripe, while pigmentation of tannin increased during this time. The results suggested that anthocyanin/pigmentation had an effect on the tannin extracted into solution. Furthermore, they show that the treated sections of the block had higher tannin activity than fruit ripened under ambient conditions. The structure variables shown to have the greatest impact on tannin activity were identified as pigmentation and molecular size. Overall, this research provides insight into berry exposure during fruit ripening and its effects on tannin structure and corresponding activity.

Funding Support: AVF

Elucidating the Fundamental Mechanism of Phenolic Extraction in Red Wine Fermentations

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The general effects of processing and fermentation on wine composition, including phenolic composition, are well studied. The variable that most influences phenolics extraction is the temperature at which fermentation is performed. Elevated fermentation temperatures (approaching 30°C) produce finished wines that are more highly colored. Similarly, ethanol production affects phenolic extraction during red wine fermentation. Based on these findings, it is likely that the fundamental mechanisms and subsequent kinetics of phenolic extraction during red wine fermentation will be greatly affected by both factors. The effects of temperature, ethanol concentration, and anthocyanin concentration on adsorption

and desorption processes of anthocyanin onto cell wall material were investigated. Independent variables included temperatures of 15 and 30°C, model wines with ethanol concentrations of 0 and 15%, and anthocyanin concentrations of 1 or 2 mg/mL. These experiments were conducted in small, benchtop solutions that mimic a single-berry fermentation environment. The desorption experiments were carried out immediately after completion of the adsorption experiments to minimize changes to the cell walls that may affect the kinetics. Results indicate that more than 90% of the anthocyanin adsorption occurs within the first 60 min of exposure to cell wall material. However, desorption appears to occur much faster: a maximum was reached after 30 min. At 15°C, different concentrations of anthocyanins produced small variations in the percentage of adsorption and desorption. At 30°C, higher anthocyanin concentrations had significantly higher adsorption rates. At both temperature conditions, increasing ethanol concentration lowered adsorption rates. The data suggests that eEthanol concentration was the driving factor of adsorption/desorption at lower temperatures, while a dynamic relationship between the three independent variables drives the processes in higher-temperature environments.

Funding Support: E & J Gallo Winery

Impact of Berry Maturity and Alcohol Content on Wine Phenolic Hydrophobicity and Content Over Time

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Polymeric pigments, which are responsible for stable wine color and wine astringency modification, are formed primarily through the reaction of anthocyanins and tannins during wine aging. In this study, cultivar, fruit maturity, and ethanol were varied (to vary initial anthocyanin and tannin content, as well as the ratio of the two) to determine their impact on polymeric pigment formation. Two cultivars (Syrah and Cabernet Sauvignon) that differ in their native anthocyanin and tannin content were harvested at three maturities: 20, 24 and 28 Brix. At each harvest, juice sugar was manipulated in the winery to simulate the other maturities. Wine samples were collected after fermentation and incubated at an elevated temperature (30°C) for four mos. Wines were sampled monthly to evaluate changes in phenolic content and hydrophobicity. Phenolic hydrophobicity is a new application of existing methods to measure tannin characteristics and activity, since tannin-protein interactions rely on hydrogen bonding and hydrophobic interactions. As tannin polymers increase in size, they become more hydrophilic, more efficient at precipitating protein, and are therefore more astringent. The range of initial wine A:T varied by cultivar and maturity (Cabernet Sauvignon: 0.36 to 0.93; Syrah: 1.3 to 2.1). Over time in both cultivars, anthocyanin content declined exponentially, while tannin content decreased only slightly. Wine phenolic hydrophobicity depended only on berry maturity and was independent of alcohol concentration. In both cultivars, ripe fruit yielded wines with significantly higher phenolic hydrophobicity, while wines from unripe fruit had the lowest phenolic hydrophobicity. For each cultivar and maturity, phenolic hydrophobic-

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ity increased as wines aged. Based on comparisons of data gathered from purified phenolic standards, our initial results suggested that the remaining phenolics in aged wine tended to be smaller. Upon further investigation, we think that loss of hydrophilic anthocyanins and tannins also helps to explain the result.

Funding Support: Wine Research Advisory Committee, Washington Wine Commission, Washington Grape and Wine Research Program, and WSU Agricultural Research Center

Quantitative Estimation of the Distributions of Iron and Copper Complexes across the pH range of Model Wines

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The renewed interest in oxidation reactions in wine has prompted a re-evaluation of the existence of free metal ions, their oxidation states, and the extent to which they form complexes with major wine constituents. The nature and extent of complex formation will vary across pH and ultimately determines the prevailing redox potential at its resting state. The redox potential establishes the starting conditions for electron transfer reactions, whether during fermentation, during aging, or in bottled wine. Using available software (HySS 4.0.31 from Hyper-Quad), the distributions of Iron (III) and Cu(II) complexes involving 16 major ligands found in wine were estimated in the pH range 2.5 to 4.5, using published complex configurations and their binding constants. The model mixture included tartrate, malate, succinate, sulfate, sulfite, chloride, nitrate, phosphate, hydroxide, gallate, caffeate, catechin, quercetin, glutathione, cysteine, and methionine as ligands in concentrations that are expected in wine and resulted in 120 entities. The results confirm that there are only small fractions of these metals in their free forms at wine pH: most are present in complexes. Ongoing work will establish how redox reactions will modify these equilibria using newly developed software and advanced experimental approaches.

Funding Support: Stephen Sinclair Scott Endowment in Viticulture and Enology

Viticulture — Remote Sensing Session

Development of a Real-Time Temperature Inversion Network for Assessing North Coast Vineyard Frost Conditions

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In grapegrowing regions that need alternatives to sprinkler frost protection, wind machines may be a viable option, provided that adequate meteorological conditions exist for their successful use. The occurrence and magnitude of temperature inversions after the onset of shoot growth are a key determinant of the potential warming effect that may be achieved by operating wind machines on frost nights. However, little information on temperature inversion conditions is available in California or elsewhere, due to the cost and difficulty of operating sufficiently tall temperature measurement towers. A previous effort by the authors used temporary 10.7 m towers to collect temperature data, but that off-line method could not provide the real-time information that would have been most beneficial to vineyard managers. In late 2013, an incipient temperature inversion-monitoring network with eight live-reporting meteorological towers was installed in vineyard areas in the Russian River watershed in Sonoma and Mendocino Counties of California. The network was gradually expanded to include 17 stations, each with temperature sensors at 1.5 m and 10.7 m to assess temperature inversion conditions at sites representative of frost-prone vineyards in the region. Sensors for wind speed, humidity, and black globe temperature were subsequently added to the stations to provide additional information on frost conditions. The black globe mimics the radiation temperature experienced by vine foliage at night, providing improved information on frost risk. The current data from these stations are reported on both NOAA and UC websites for public access. This effort has demonstrated the value of providing detailed temperature inversion and other spring frost-related information to vineyard managers who must make critical decisions regarding their frost protection strategies.

Funding Support: NOAA Russian River Habitat Blueprint

A Thermal Camera-Based Smartphone Application to Measure Vine Water Status

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The concept of using canopy temperature to estimate plant water status was developed during the 1970s. Unfortunately, this technique has not been widely adopted due to the high cost of equipment and the need for office-based image analysis. Smartphones have several advantages over specialist monitoring systems including ubiquity, price, user familiarity, and ease of implementing updates. They also contain sufficient computing power that analysis and support software can be contained within the handset. Thermal cameras that connect to smartphones have recently been released by several vendors. One of these (FLIR-One®) was evalu-

Viticulture — Remote Sensing Session – CONTINUED

ated using an irrigation trial on Chardonnay and Cabernet Sauvignon grapevines in the Riverland region of South Australia. Crop water stress index (CWSI) was calculated based on the canopy temperature relative to canopies that are well-irrigated or under severe water stress (represented by artificial reference leaves). Images were collected from the shaded side of the canopy so that the system could potentially be used under a range of environmental conditions. After 10 days of assessment over a range of irrigation levels, measurements collected with the thermal camera were correlated with stem water potential ($r^2 \approx 0.6$) and stomatal conductance ($r^2 \approx 0.7$). Under warm and clear conditions, the relationship was stronger ($r^2 \approx 0.8$ for stomatal conductance), but windy and humid conditions disrupted both CWSI and the reference measurements. To test the utility of this system for assessing vine water stress and making irrigation decisions, a smartphone application was developed for user acceptance testing. The application was designed to automatically select the wet and dry reference leaves and the canopy from the thermal image, making it easy to calculate the CWSI in the vineyard. User feedback has been positive and the final version of the application will be released for the 2017 to 2018 season.

Funding Support: Wine Australia

THURSDAY ORAL ABSTRACTS

Estimating Vine Evapotranspiration Using Multispectral and Thermal Sensors Placed aboard an Unmanned Aerial Vehicle

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A field experiment was carried out to implement a remote sensing energy balance (RSEB) algorithm to estimate spatial variability of vine water requirements or evapotranspiration (ET) over a drip-irrigated Cabernet Sauvignon vineyard located in the Pencahue Valley in the Maule Region, Chile (35°25′ LS; 71°44′ LW; 90 m asl). For this study, a helicopter-based unmanned aerial vehicle (UAV) was equipped with multispectral and infrared thermal cameras to obtain simultaneously the normalized difference vegetation index (NDVI) and surface temperature ($T_{surface}$) at very high resolution (6 cm × 6 cm). Meteorological variables and surface energy balance components were measured at the time of the UAV overpass, near solar noon. The performance of the RSEB algorithm was evaluated using measurements of ET from an eddy correlation system. The RSEB algorithm overestimated ET by ~13% with a root mean squared error and mean absolute error of 0.43 and 0.29 mm/day, respectively. Major errors were associated with the estimation of sensible heat flux from the canopy and soil, especially when the wind speed was greater than 2.5 m/sec. Results demonstrated that multispectral and thermal cameras placed on an UAV could provide an excellent tool to evaluate the intravineyard spatial variability of ET.

Funding Support: Chilean National Science Foundation

Bold type indicates presenting author

Viticulture — Remote Sensing Session – CONTINUED

"To Fly or Not to Fly": Airborne Remote Sensing for Determination of Vine Water and Nitrogen Status at a Regional Scale

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In the face of a warming climate, the long-term sustainability of Australian vineyards depends on prudent management of natural resources such as freshwater for irrigation. Likewise, nitrogen (N) fertilizers aid vine growth and improve juice nitrogen levels for healthy fermentations, however, excess N applications in vineyards can result in excessive vine vigor, leading to increased water use and leaching from vineyards. Recently, remote sensing platforms have emerged to characterize the water and nitrogen status of vineyards at high spatial resolution (<0.35 m), allowing viticulturists to make decisions on water and fertilizer applications at the sub-block level. Remotely-sensed water status is typically based on thermal imagery, or thermography, while nitrogen status is based on multispectral imagery to determine specific vegetation indices that relate to leaf nitrogen concentration. Our objectives were: (i) to validate vine water and nitrogen status data obtained from an airborne remote sensing platform using conventional on-ground measurements across an entire viticultural region; and (ii) to determine whether remotely-sensed vine water status data could be used to make irrigation scheduling decisions. Airborne campaigns were conducted over several timepoints in the 2015 to 2016 and 2016 to 2017 seasons in the Coonawarra region of South Australia. Remote sensing was a powerful tool for large-spatial scale characterization of water and N status of Cabernet Sauvignon and Shiraz grapevines and generally correlated well with ground-truthed data. Remote sensing also improved irrigation uniformity by delineating zones of non-uniformity in certain blocks. Temperature indices of vine water status varied based on cultivar and phenological stage and, therefore, require calibration over multiple seasons to be useful for irrigation scheduling.

Funding Support: Coonawarra Grape and Wine Inc.

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Industry

Performance of Wine Tank Anchorage Systems in the Marlborough Earthquakes

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Recent earthquakes affecting the Marlborough wine region in New Zealand have shown a disconnect between winery infrastructure design and desired seismic performance. Current design codes focus on life safety, but not protection of tank contents or the infrastructure asset. Subsequently, Marlborough wineries have had to respond to significant damage to tanks and associated services, business disruption, and potential loss of market share. Facilities with the OnGuard anchorage system fitted to their tanks were spared these issues, as the ductile and holistically designed anchor protected the tank and allowed wineries. Furthermore, the innovative design of the anchor allows simple retrofit to most tanks and is rapidly been installed to older tank stock throughout the Marlborough region.

Funding Support: OnGuard Limited

New Innovations in the Use of Ozone in Wineries

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Since the early 90's, ozone has been used by wineries for many sanitation applications (i.e., barrels, tanks, bottling lines, etc.). Application protocols are available for these applications. The recent need to reduce chemical inputs, water, and labor while controlling mold, odors, and vectors has extended ozone use into many other winery applications. Using ozone gas for barrel storage, mold, odor, and fruit fly control is now common practice. Some side benefits of ozone gas for these applications has included improved wine quality from barrels, reduced topping losses, and reduced water use. In addition, using pressurized, cold ozonated water for barrel cleaning and sanitation and the availability of ozone-compatible tank cleaners reduces labor costs and water use by up to 50%. The presentation will discuss both the historic use of ozone in wineries and the newest innovations. The environmental benefits, cost savings, and product quality improvement will also be covered.

Funding Support: McClain Ozone

Industry – CONTINUED

A Review of Plastics Use in Winemaking: Permeability and Sorption Considerations

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Plastic materials may contact wine during all stages of winemaking: fruit transportation, crush, and storage involve all manner of equipment and materials like pumps, hoses, fining agents, and filtration for final packaging. Plastics may offer numerous benefits, including cost savings, desirable functionality, or both. However, potential consequences of using plastic include sorption and permeability. Sorption occurs when wine constituents are lost from solution to the plastic phase, while permeability is the rate at which a gas or vapor passes through a polymer. Ethanol and aroma compounds may be lost via sorption or permeation of wine volatiles into the film, which may impact wine quality. Both sorption and permeability depend on which plastic polymer the wine contacts, the molecular size of the volatile compounds present, and storage conditions. This work reviews current research on the effects of grapes and wine interacting with plastics during winemaking, including during processing and storage. Additionally, this work compares gas permeability of plastics to micro-oxygenation techniques, wood, and barrels, so winemakers can better understand and choose among materials to use during winemaking.

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Enology and Viticulture

Red Blotch Disease Alters Grapevine Primary Metabolism Resulting in Unstable Berry Flavonoid Composition

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Grapevine red blotch-associated virus (GRBaV) is a major threat to grape and wine industries in the United States, impacting vine health and berry composition. Cabernet Sauvignon/110R vines grown in Oakville, CA, were grouped as healthy or symptomatic during the 2015 growing season and, using qPCR and primers specific to the GRBaV genome sequence, vines were classified as GRBaV (-) or GRBaV (+) in 2016. We measured leaf gas exchange, midday stem water potential, components of yield, and berry composition at five time points during the 2016 growing season. Berry flavonol and anthocyanin composition was characterized with C_{18} reversed-phase HPLC. Midday stem leaf water potential was consistently greater in GRBaV(+) vines. Net carbon assimilation was consistently lower in GRBaV(+) vines, but recovered postharvest. Likewise, stomatal conductance and evapotranspiration were reduced by 40% from DOY 230 to 265 for GRBaV(+) vines and did not recover until one week postharvest. Conversely, intrinsic water use efficiency of GRBaV(+) vines was similar to GRBaV(-), with the exception that they were 42% greater one week postharvest. Components of yield were not affected by the presence of GRBaV. However, the Brix of GRBaV(+) vines were 18% lower compared to GRBaV(-). Conversely, titratable acidity of GRBaV(+) was 18% greater than GRBaV(-). The glucoside, acetylated, tri-hydroxylated, and coumarated forms of anthocyanidins were reduced by 20% in GRBaV(+) vines. Likewise, the flavonols and their tri-hydroxylated forms were reduced in similar amounts with GRBaV(+). The results provide evidence that GRBaV infection may induce significant reduction in primary metabolism, reducing carbon assimilation and stomatal conductance and altering berry and flavonoid composition.

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Sanitary Status of Grapevine Nurseries in Washington State

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Virus-tested plant material is recognized as the best antidote for establishing healthy vineyards. With increasing demand for "clean" plants, there is a critical need to maintain virus-tested plant material in certified grapevine nurseries to ensure that viruses are not disseminated via plant material from nurseries to vineyards. With this objective, we have collaborated with grapevine nurseries and the Washington State Department of Agriculture to implement high sanitary standards in Certified Mother Blocks. We have adopted a composite sampling strategy for high-throughput virus indexing of leaf samples collected during the season and cane samples collected during the dormant season. Extracts from composite

samples were tested initially using molecular diagnostic assays for the presence of *Grapevine leafroll-associated virus 3* (GLRaV-3) and *Grapevine red blotch-associated virus* (GRBaV). In 2016, 2054 composite samples collected from different grapevine cultivars maintained by three nurseries were tested for GLRaV-3 and GRBaV. Names of nurseries were withheld due to confidentiality. Based on virus indexing results, only 47 of the 2054 composite samples (~2.29%) tested positive for GLRaV-3. All 2054 composite samples tested negative for GRBaV. A subset of these composite samples was subjected to high-throughput sequencing to confirm the above results and document the presence of other viruses. Testing composite samples, followed by retesting samples from individual vines if a composite sample comes up positive, was advantageous for virus-indexing large numbers of samples. Results obtained during the past three seasons showed the absence of GRBaV in grapevines maintained in Certified Mother Blocks. However, the spread of GLRaV-3 into Mother Blocks is a concern for nurseries. Therefore, virus indexing of grapevines in Certified Mother Blocks at regular intervals is necessary to maintain high sanitary standards in nurseries and to make "clean" plant material available to growers.

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Defoliation of Grape Leaves Associated with Downy Mildew, Anthracnose, and *Isariopsis* Leaf Spot

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Downy mildew, anthracnose and *Isariopsis* leaf spot are serious diseases in viticulture that can drastically reduce production during heavy infections. Weather affects pathogen development and can promote epidemics, with effects up to abscission of the affected organs. However, the effects of cultivar, rootstock, weather factors, other diseases, and defoliation are not well known. From 2011 to 2013 (two growing seasons), 10 leaves of one shoot per plant in six vines of each combination of cultivar and rootstock were monitored from budbreak until fall, for 540 leaves. Survival analysis was used to investigate the influence of factors on survival of leaves. The Kaplan-Meier estimator showed the proportion of grape leaves surviving to a specific time. The estimated median leaf survival time was 117 to 170 days (season 1) and 99 to 153 days (season 2). Survival analysis showed a significant increase in the risk of defoliation due to high relative humidity, rain, leaf wetness, and temperature. The effects of the monitored diseases posed highly significant increased risk of defoliation and their influence varied with cultivar and rootstock. Later cultivars had shorter leaf survival times than earlier cultivars, influenced by the inoculum level. *Isariopsis* leaf spot had less influence on leaf fall than the other two diseases.

Funding Support: CAPES

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Map-Based Positional Cloning of Powdery Mildew Resistance Genes from the Chinese Grape Species *Vitis piasezkii*

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All cultivated grape varieties are highly susceptible to powdery mildew (PM), a fungal disease caused by the pathogen *Erysiphe necator*, which can evolve rapidly under high selection pressure. The wild Chinese grape species, *Vitis piasezkii,* is resistant to PM and major resistance loci *Ren6* and *Ren7* were identified on chromosomes 9 and 19, respectively. *Ren6* confers complete resistance, while *Ren7* provides partial resistance. In this study, we report a refined positioning of the *Ren6* locus and the initial results of *V. piasezkii*-based BAC library screening. A total of 38 recombinant plants from backcross populations were selected with flanking SSR markers. Plants were evaluated for PM resistance and based on both genotypic and phenotypic scores, the new position of the *Ren6* locus is between markers PN9-66.01 and PN9-068. We developed a BAC library from the genomic DNA of the F1 seedling 11373-087, which has an average insert size of ~110 Kb and seven to eight X coverage. DNA probes (600 to 700 bp in length) in close proximity to the flanking markers and without repetitive regions were designed using the reference grape genome PN40024. The BAC library will be screened and clones will be identified and sequenced to develop a physical map of the *Ren6* locus capable of identifying candidate powdery mildew resistance genes. Comparisons will be made with the susceptible reference grape genome and the Cabernet Sauvignon genome for this region. This study will be the first attempt to identify candidate resistance genes in *V. piasezkii*. Candidate genes will be tested in a susceptible *V. vinifera* cultivar(s) to verify their ability to confer resistance.

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

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Grapevine red blotch-associated virus (GRBaV) was identified as the causal agent of red blotch disease in 2011. Berries from infected grapevines can present lower sugar concentrations than healthy grapes. For the last 3 years the impacts of red blotch disease on grape and wine composition have been investigated and results indicate that grape berries from symptomatic Cabernet Sauvignon, Chardonnay, and Merlot vines mostly have a decrease in Brix and anthocyanin concentrations, with variable impacts on other phenolic compounds such as flavan-3-ols and their polymers when compared to fruit from healthy vines. The difference in sugar concentration at harvest resulted in significantly higher ethanol concentrations in wines made from fruit from healthy vines in 2014 and 2015, which strongly af-

fected sensory properties. This research compared grape and wine composition of symptomatic and healthy vines harvested on the same date and additional symptomatic vines harvested at a later date at the same Brix as the healthy fruit. Berries from Cabernet Sauvignon, Chardonnay, and Merlot from Napa Valley, Sonoma County, and San Luis Obispo, respectively, were collected weekly from *veraison* until harvest and analyzed for standard chemical composition (Brix, pH, TA, sugar loading, and malic acid) and phenolic composition by protein precipitation assay and RP-HPLC. Wines made from fruit produced on healthy and symptomatic vines, and fruit harvested from symptomatic vines on a subsequent date, were made in triplicate. Results confirmed that grapes from symptomatic vines had decreased sugar accumulation and increased TA during ripening in all three varieties. Wines made with grapes from symptomatic vines harvested at the same Brix as healthy vines showed less impact of the disease, producing wines more similar in anthocyanin and tannin concentration to wines made from healthy fruit.

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Complete Genome Sequence of *Grapevine Leafroll-Associated Virus 1* Isolates from Washington Vineyards

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Five distinct species of closteroviruses, designated as *Grapevine leafroll-associated virus 1*, *2*, *3*, *4,* and *7* (GLRaV-1, -2, -3, -4, and -7) have been reported in grapevines (*Vitis vinifera*) symptomatic or suspected of leafroll disease infection. The complete genome sequence has been determined for other GLRaVs and their strains, except for GLRaV-1. We determined the complete genome sequence of GLRaV-1 isolates from Washington vineyards. The genome of GLRaV-1 from Chardonnay (WA-CH) and Pinot noir (WA-PN) was amplified into four overlapping cDNA fragments using sequence-specific primers. The derived cDNA clones specific to each fragment were sequenced in both orientations by 'primer walking' using progressive sequence-specific primers. The 5'- and 3'-terminal sequences were determined using the RACE system. The sequences were annotated and assembled into a complete genome of 18,731 and 18,946 nucleotides, respectively, for WA-CH and WA-PN isolates. Both isolates showed similar genome organization, encoding nine putative open reading frames and with unusually long non-translated regions (NTRs). The most striking feature of GLRaV-1 isolates is a large 5' NTR with a variable number of ~65 nt tandem repeat sequences. Differences in the 5'NTR sequences of GLRaV-1 isolates was exploited to develop a RT-PCR-based RFLP assay for discriminating virus isolates into three distinct variant groups. Northern blot hybridization of total RNA from virus-infected grapevines with gene-specific riboprobes revealed the presence of sub-genomic RNAs (sgRNAs) corresponding to the coat protein (CP) and ORFs p21 and p24, with p24 sgRNA present at relatively higher levels than other sgRNAs. The 5' termini of sgRNAs corresponding to the CP, CPd1, CPd2, p21, and p24 were mapped to the virus genome and the leader sequence for these sgRNAs determined. The

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results provide a foundation for further elucidation of the comparative molecular biology of GLRaVs.

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Feeding and Reproductive Hosts of the Three-Cornered Alfalfa Hopper, a Vector of *Grapevine Red Blotch-Associated Virus*

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Grape must from vines infected with *Grapevine red blotch-associated virus* (GRBaV) exhibit negatively altered chemical properties and decreased Brix, collectively resulting in reduced wine quality. Recently, the three-cornered alfalfa hopper (3CAH) was determined to vector GRBaV in a greenhouse study. Before this discovery, limited research had been conducted on 3CAH in California vineyards due to their previous status as only an incidental pest. As a result, there is limited information regarding 3CAH feeding and reproductive hosts in Californian vineyards. I hypothesize that the 3CAH is attracted to vineyards by certain feeding and reproductive weed/cover crop hosts. To test the foundation of this hypothesis, 10 weed species associated with vineyards and 15 commonly planted cover crops were individually caged in the greenhouse with six 3CAH adults (three female and three male) and replicated four times. Feeding and reproductive hosts were determined. Data obtained from this research can be used to generate a list of weeds and cover crops that support 3CAH and an annual succession map to assist growers in management decisions.

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Impacts of Grapevine Leafroll and Red Blotch Diseases in Commercial Vineyards

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Grapevine leafroll (GLD) and red blotch (GRBD) are distinct viral diseases affecting vineyards in many grapegrowing regions in the United States. This study was conducted during several seasons to assess impacts of GLD and GRBD in red-fruited winegrape (*Vitis vinifera*) cultivars planted in commercial vineyards. Grapevines with and without symptoms were identified for each cultivar and tested for the presence of *Grapevine leafroll-associated virus 3* (GLRaV-3) and *Grapevine red blotch-associated virus* (GRBaV) to ensure symptomatic vines were positive for either GLRaV-3 or GRBaV and non-symptomatic vines were negative for the two viruses. At least 20 vines showing symptoms and testing positive for GLRaV-3 or GRBaV and an equal number of vines without symptoms and testing negative for the two viruses were selected for each cultivar. In each season, data on fruit yield was collected from each cultivar at the time of commercial harvest. Similarly,

data on fruit quality attributes (total soluble solids measured as Brix, juice pH, titratable acidity [TA], and total anthocyanins) was compared in grapes harvested from vines with or without symptoms. Both GLD and GRBD affect fruit yield and quality (especially sugars) in red-fruited winegrape cultivars examined during the past two to three vintages. The negative impacts of GRBD on fruit yield and quality appeared to be relatively higher than GLD in cultivars examined in this study. Interestingly, impacts on grape juice pH and TA were not as pronounced as impacts on fruit yield and berry sugars. Mixed results were obtained with berry anthocyanins, depending on the disease and cultivar. The negative impacts of GLD and GRBD on fruit yield and berry sugars were variable among cultivars and across vintages, suggesting cultivar- and season-specific differences to viral infections.

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Effect of Pruning on Grapevine Shoot and Cluster Development as a Function of Arm Position along the Cordon

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Cordon-trained, spur-pruned grapevines frequently exhibit non-uniform, developmentally delayed shoots at mid- and head-arm positions. The variation in vine growth, cluster development and ripeness impacts the timing and success of vineyard management activities such as leaf and cluster thinning and harvest. The objective of this research is to determine the role pruning severity and resulting shoots/meter have in minimizing developmental variation at the head-, mid- and end-shoot positions on a vine. The study was conducted in Oakville, California, on bilateral cordon-trained, spur-pruned Cabernet Sauvignon vines on 1.8 × 2.5 meter spacing. Vines were pruned to either 5.5 or 11.1 shoots/meter for a total of 12 or 24 buds per vine, respectively. Parameters tracked over the course of the season included shoot length, internode length and diameter, and pruning and cluster weights. In addition, berry chemistry analysis (soluble solids, pH, TA, and total phenolics) was performed at harvest. Shoots originating from arm positions at the end of cordons tended to be stronger sinks than those at mid and head positions. Specifically, fewer shoots/meter homogenized shoot internode length, diameter, and berry chemistry. The results of this study will be used to make pruning recommendations to address non-uniformity in vine growth and will serve to optimize vine balance and homogenize cluster production along the cordon arm.

Funding Support: California State University Agricultural Research Institute

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Specific Yeast-Derived Foliar Spray Application Affects Anthocyanin Gene Expression and Accumulation in Sangiovese

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Biotic and abiotic elicitor applications can activate secondary metabolite biosynthetic pathways, increasing accumulation of phenolic compounds, particularly anthocyanins. This research examines the effects of applying a commercial foliar spray, LalVigne™ MATURE, a formulation of 100% natural, inactivated wine yeast (*Saccharomyces cerevisiae*), on berry skin anthocyanin accumulation in uniform potted Sangiovese vines. To determine the molecular mechanisms underlying the action of LalVigne™ MATURE, its impact on expression of anthocyanin biosynthetic pathway genes was evaluated. In 2016, LalVigne™ MATURE was applied twice (at the beginning of veraison and nine days later, when veraison was over 70% in all vines), covering the canopy and bunches of three different vines (LVM). Ripening parameters and anthocyanin accumulation were compared with those of three untreated control vines (C). Sugar accumulation increased after veraison, with similar trends for C and LVM berries until harvest and no differences in Brix, pH, or titratable acidity. Anthocyanin accumulation was significantly increased in LVM berries, which showed higher levels of these compounds after the first foliar application of the specific yeast derivatives in comparison with the untreated C. These preliminary results show that LalVigne™ MATURE foliar spray increased anthocyanin accumulation in the Sangiovese grapes, independently from sugar accumulation. Preliminary gene expression analyses on the main biosynthetic anthocyanin genes, UFGT and VvMYBA1, showed more expression in LVM berries after the treatment in comparison with the untreated C.

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Bunch Exposure Management in a Challenging Environment Using Sun Protection Agents

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Recent warming trends and erratic weather patterns have increased the incidence of sunburn injury to grape berries, necessitating the use of sun protection agents. The objective of this study was to determine the effects of kaolin (Surround WP) and calcium carbonate on fruit composition, including aroma characteristics, in Cabernet Sauvignon. These sun protection agents were applied just after fruit set at the manufacturer's recommended dosage until runoff using a backpack sprayer over the fruit zone and the whole canopy. Vines and shoots without the agents served as untreated controls. Sunburned berries showed degradation of crystalline wax structure, while such structures remained intact in clusters sprayed with sun protection agents. Sunburned berries of all cultivars dehydrated, concentrating the TSS, reducing fermentable sugars, potassium, and organic acids and increasing pH. Among the phenolic compounds, total anthocyanins and amount of individual anthocyanins were reduced significantly, while quercetin levels increased in sunburned berries. The levels of various volatile aroma compounds such as ter-

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penoids, C13-norisoprenoids, and C6 compounds varied among treatments. The composition of clusters sprayed with sun protection agents was not compromised, while both kaolin and $CaCO_3$ showed similar effects.

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Crop Load Management to Improve Ripening and Aromatic Contents in White Grapes in the Okanagan Valley

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Crop load management by cluster thinning is a viticultural practice that can improve ripening and the concentrations of secondary metabolites, including volatile organic compounds. Little work has been done on white grapes in the Okanagan Valley. The timing of cluster thinning can alter the efficacy of the treatment. In this study, field-grown Gewürztraminer were cluster-thinned post-fruit set and at veraison at two levels: light (10 tonnes/ha) and moderate (15 tonnes/ha). A control treatment (20 tonnes/ha) was maintained for comparison. The hypothesis is that reducing crop load via cluster thinning will stimulate ripening and synthesis of beneficial volatile compounds, with early thinning application having a more positive effect than late application. Treatments were replicated on five plots arranged in a randomized block design. Sampling occurred every seven to 14 days, starting three weeks after fruit set. The effect of these treatments on photosynthesis, vegetative growth, and sugar, acid, and volatile compound concentrations was analyzed. Sugar concentration and vine yield were significantly affected by cluster thinning; however, the timing of cluster thinning seemed to be less important. Previously identified genes involved in central and specialized metabolism were quantified and analyzed for treatment response. Free and bound volatiles were quantified through solid-phase micro-extraction gas chromatography mass spectroscopy. The goal of this project is optimize Gewürztraminer grape quality through targeted crop load management practices, as volatile organic compounds are closely tied to the economic potential of wine grapes.

Funding Support: British Columbia Investment Agriculture Foundation, British Columbia Wine and Grape Council

Irrigation Management for Improving Ripening and Aromatic Contents in White Grapes in the Okanagan Valley

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Deficit irrigation of grapes can decrease berry weight and increase concentrations of secondary metabolites, including the volatile organic compounds. This practice is often used in red grape varieties to improve grape and wine quality; however, little work has been done with white grapes, particularly in the Okanagan Valley. The timing of deficit irrigation application can alter the efficacy of the

treatment. In this study, field-grown Gewürztraminer under a deficit irrigation regime applied at different periods throughout berry development (well-watered, preveraison deficit, postveraison deficit, and prolonged deficit) were characterized for their ecophysiological, biochemical, and molecular response to the treatments. The hypothesis was that application of deficit irrigation will induce synthesis of quality-determining volatile compounds, with preveraison and prolonged application of water deficit being more effective than later application. Treatments were replicated on four plots arranged in a randomized block design. Sampling occurred every seven to 14 days starting three weeks after fruit set. Grapevine leaf water potential was monitored using the Scholander pressure chamber at ±1 hr from solar noon. The effect of these treatments on leaf water potential, photosynthesis, vegetative growth, and sugar, acid, and volatile compound concentrations was analyzed. Ecophysiological parameters, sugar concentration, berry weight, and vine yield were significantly affected by deficit irrigation; however, there was a clear effect from timing of application as well. Previously identified genes involved in drought response and central and specialized metabolism will also be analyzed for treatment response. Free and bound volatiles will be quantified using solid-phase micro-extraction gas chromatography mass spectroscopy. The goal of this project is optimize Gewürztraminer grape quality through targeted irrigation practices, as volatile organic compounds are closely tied to the economic potential of winegrapes.

Funding Support: British Columbia Investment Agriculture Foundation, Mitacs, British Columbia Wine and Grape Council

Effect of Liquid Fertilizer on Growth, Quality, and Anthocyanin Levels of Grape Berry

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Liquid fertilizer (N:P:K=0:5:4) containing yeast cell extract improves plant physiological activity. The increase in diameter, weight, and Brix at the end of stage II, immediately before veraison, in treated Muscat of Alexandria berries occurred one week earlier in fertilized than in untreated berries in 2015. The temporary increase of indoleacetic acid (IAA) level in berry after treatment may cause the accelerated changes from stage II to stage III of berry development. However, there were no differences in berry diameter, weight, Brix, and titratable acidity (TA) content at harvest between treated and untreated berries. Berry diameter and weight of Harmo noir (Cabernet Sauvignon × Zweigelt) treated with the fertilizer during growth stages I and II increased in 2016, but there was no difference in Brix or TA between treated and untreated berries. Berry diameter of Merlot fertilized at the end of growth stage II and at early growth stage III in 2016 increased, but there were no differences in berry weight, Brix, and TA between treated and untreated berries. Anthocyanin levels also increased. The temporary increase in IAA level at the end of growth stage III occurred seven days earlier as a result of the treatment. We conclude that the liquid fertilizer treatment might have stimulated grape berry enlargement and coloring.

Funding Support: Meijo University

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Timing of Leaf Removal Differentially Affects the Sensory Quality of Red and White Wines

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Riesling and Sauvignon blanc wines from the Okanagan Valley often have a crisp, fruit-forward style with attributes like stone fruit, tropical fruit, citrus, spice, floral, and honey. Characteristic descriptors for Shiraz wines of this region are dark fruit, black cherry, cooked fruit, pepper, and smooth tannins. Three experiments evaluated fruit maturation and quality and wine sensory quality with respect to timing and amount of leaf removal. The experiments were a randomized complete block design with five treatments and four blocks. Leaf removal treatments applied in the fruiting zone were: 100% at fruit set or veraison, 50% at fruit set or veraison, and no leaf removal. The vines had spur-pruned bilateral cordons with vertical shoot-positioning. Basic composition and phenolics were measured in fruit and wine. The blind sensory evaluations were by 16 judges who tasted 10 wines twice in a balanced, incomplete block design. In Shiraz, leaf removal at fruit set compared to at veraison and no leaf removal resulted in lower vegetative but higher dark fruit and pepper flavor and aroma in wines. Astringency and phenolics were higher with early and 100% leaf removal. In Riesling and Sauvignon blanc, the wines benefited from later leaf removal, showing more tropical and citrus fruit flavor and aroma when applied at veraison than at fruit set.

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

Effects of Irrigation Deficit Timing on Vine Water Stress and Winter Hardiness in Merlot

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The effects of irrigation deficit timing on yield, fruit quality, and winter hardiness of Merlot in the Okanagan Valley of British Columbia were studied to explore the relationship between seasonal photosynthetic rates and bud hardiness. The irrigation treatments were full irrigation (FI), which was a sustained, mild-deficit regime, and three treatments with a severe irrigation deficit period: prebloom-to-harvest (PBH), fruit set-to-veraison (FSV), and fruit set-to-harvest (FSH). Leaf gas exchange and stomatal conductance were less in vines undergoing severe deficit irrigation. Yield was greater for FI than PBH vines, which had fewer and smaller berries per cluster. Compared with all severe deficit treatments, FI produced larger berries, lower juice pH, and higher juice TA, but similar juice soluble solids. Bud hardiness in early November was improved by FSV over FI, but sustaining the severe deficit period beyond veraison (FSH and PBH) did not improve hardiness compared with FI. Enhanced bud hardiness associated with higher photosynthesis rates found previously was not observed in this study, indicating that in Merlot, vine water stress and carbon balance less-consistently influence bud winter hardiness than yield components and fruit quality.

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

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Shifts in Proanthocyanidin Composition of Cabernet Sauvignon Are Modulated by Selective Shading and Water Deficit

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Interactive effects of selective cluster shading and water deficits on proanthocyanidin content and composition of Cabernet Sauvignon grapes grown in Oakville, CA, were investigated, aiming to mitigate the deleterious effects of solar overexposure. The experiment used three colors of shade nets with an untreated control and two applied water amounts arranged factorially in a split-plot design with four five-vine replicates. The three colors of shade nets (black, aluminet, and pearl) were placed onto the canopy on both sides (1000 mm) adjacent to the fruiting zone at fruit set. Two water deficits were applied starting at fruit set: a sustained deficit irrigation treatment (SDI) received 65% of estimated crop evapotranspiration (ET_c) and regulated deficit irrigation (RDI) received 25, 65, and 65% ET_c at fruit-set to veraison, veraison to harvest, and harvest to leaf fall, respectively. Primary metabolism (net carbon exchange, transpiration, stomatal conductance, intrinsic water use efficiency, mid-day leaf water potential, components of yield) was assessed every 21 days and secondary metabolism (berry skin and seed proanthcyoanidins) were assessed six times starting at 4 Brix. Proanthocyanidins were characterized by acid-catalysis in the presence of excess phloroglucinol, followed by reversed-phase HPLC. Water deficits resulted in transient stress on the grapevines that received RDI between fruit set and veraison. Net carbon exchange, stomatal conductance, and intrinsic water use efficiency were not affected consistently. Although berry mass showed a significant increase with shade nets, water deficits did not affect it. Aluminet had the least yield than the other treatments. Mean degree of polymerization (mDP) of skin proanthocyanidins was not affected, but seed tissue mDP of black and red nets under SDI and blue nets under RDI decreased significantly. The results indicate that colored shade nets may negatively impact yield and, in combination with SDI or RDI, tactile sensation through decreased mDP in seeds.

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Relationship of Soil Moisture and Leaf Water Potential in Deficit-Irrigated Tempranillo Grapes: A Working Hypothesis

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The purpose of the study was to develop a regulated deficit irrigation program as a strategy to optimize water use on Tempranillo grapes in the Temecula Valley of California and document the effect of deficit irrigation on juice composition. We are testing the hypothesis that the relationship between soil moisture and leaf water potential in Tempranillo grapes receiving deficit irrigation can be modeled. Three deficit irrigation scheduling treatments, replicated three times, were assigned to blocks of 150 vines in a complete randomized block design. The deficit irrigation treatments were T1: sustained deficit at 50% ET from bud-

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break through veraison; T2: postveraison deficit at 10% ET at veraison; and T3: preveraison deficit at 10% ET beginning at fruit set. The vines from all treatments were irrigated at 100% ET after harvest. Leaf water potential was measured weekly using a pressure chamber. In most cases, at the time irrigation events took place, the pressure chamber readings were between 11 to 13 bars. The correlation between the water potential and soil moisture was analyzed at 4-inch increments up to 36 inches below the soil surface to determine if soil moisture levels can indicate the stress level of a grapevine. The analysis revealed a negative correlation between leaf water potential and the previous day's soil moisture at 36 inches below the surface for postveraison and preveraison treatments, while the sustained deficit at 50% ET showed no identifiable relationship. Future research will focus on modeling this relationship to develop a practical deficit irrigation guide based on measureable soil humidity.

Funding Support: California Department of Food and Agriculture

Use of Infrared Temperature Sensing to Continuously Measure Grapevine Water Status for Irrigation Scheduling

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Grapevines in arid and semi-arid viticultural regions rely on supplemental irrigation to maintain growth and productivity. Fresh water is often scarce in such regions globally and increased irrigation efficiencies are continually sought. Measurement of vine water status is a key approach to improving irrigation efficiency in vineyards. Under certain environmental conditions, leaf/canopy temperature and associated temperature indices are closely related to plant water status or stress. The concept of using non-contact infrared temperature sensing as a tool to manage irrigation is not new; however, the development and realization of a practical tool for growers based on this concept has been hampered by the high cost of existing temperature-sensing platforms. Here, we present a new, low-cost platform for continuous measurement of vine water status based on the Arduino™ microcontroller and miniature infrared thermometers. This platform has high temporal resolution, small form factor, local data storage and wireless data transfer capabilities, the ability to integrate additional environmental sensors, and low cost. We have tested this platform with a new simplified index of plant water status based on canopy temperature under a range of vine water status conditions and compared this index to existing temperature indices. Predictive models of vine water status and decision support systems can be integrated into this platform for automated irrigation scheduling in vineyards.

Funding Support: Coonawarra Grape and Wine Inc.

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Maintaining Vineyard Production with Season-long Deficit Irrigation

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Subsurface microirrigation using plastic tubes to deliver water directly into the lower root zone was examined over two growing seasons (2015 and 2016) in the Red Mountain AVA of Washington State to determine advantages over surface drip irrigation in water conservation to maintain vineyards with season-long deficit irrigation. Irrigation was applied on the same schedule used to achieve production and quality goals in a commercial planting of Cabernet Sauvignon; however, total water applied via subsurface delivery was reduced to 60, 30, or 15% of the surface drip amount. Continuous and pulse delivery was compared for the direct root-zone (DRZ) applications, but no differences were attributed to either method. Additionally, no consistent differences correlated with a particular water delivery depth below surface. A randomized block design with three replicates was used to determine statistical differences in fruit production and chemical composition of harvested grapes. In 2015, the hottest and driest season on record, DRZ treatments receiving reduced irrigation season-long yielded from 90 to 70% of the grape production of surface drip application. In 2016, commercial production was 50% higher than in 2015, and DRZ plot production was slightly lower than in 2015. In 2015, clusters from each treatment and commercial block were collected during veraison and harvest weights per vine were made in late-September. Harvest weights were taken during commercial harvest in 2016 and replicated samples of DRZ and commercial plots were submitted to a private lab for a full grape analytical panel. Acidity diminished with increasing water reduction, while Brix, tannins, and anthocyanins increased progressively with water reduction. Results to date show promise for maintaining vineyard production using DRZ, with concomitant increase in grape quality while conserving water resources during years of limited supply.

Funding Support: Washington Winegrowers, Northwest Center for Small Fruit Research, WSDA Specialty Crop Block Grant Program

Production Systems and Applied Water Amounts Interact on Productivity and Berry Composition of Zinfandel

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A field trial was conducted in the hot climate of central California to assess the interactive effects of production systems and applied water on canopy architecture, yield, water footprint, and berry composition of Zinfandel. Production systems were split-canopy, cane-pruned (CP, six-canes with eight nodes), spur-pruned (SP, 22 two-node spurs), and mechanically box-pruned single high-wire (MP, 100 mm hedge). Applied water treatments were sustained deficit irrigation (SDI), receiving 80% of crop evapotranspiration (ET_c) from budbreak to leaf fall, and regulated deficit irrigation (RDI), receiving 80, 50, and 80% of ET_c between budbreak and fruit set, fruit set to veraison, and veraison to leaf fall, respectively. The vineyard

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received 87, 30, and 46% of the 10-year average of winter precipitation in years 1, 2, and 3 of the study. The Y_l of SDI was consistently greater, while production systems did not affect it. The yield of SP and CP declined consistently while MP did not. The leaf area to fruit ratio of MP in combination with SDI was maintained at 0.70 m^2/kg, while SP and CP varied greatly during the study period. RDI reduced the water footprint of Zinfandel regardless of production system, but the associated decrease in yield was not commercially acceptable. The water foot print of MP was 22% less than traditional production systems. Our results provide evidence that the combination of MP and SDI is a sustainable production system based on sustained yield, optimum leaf area to fruit ratio, and reduced water footprint in a hot climate.

Funding Support: American Vineyard Foundation

Using Mass Balance to Determine Plant Water Status of Individual Grape Vines

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Bulk irrigation is the status quo in vineyards. However, even when comparing vines planted side by side, irrigation needs may vary dramatically. Additionally, berry quality may be improved by decreasing this heterogeneity between vines. For these reasons, along with the increasing scarcity of water resources in California grapegrowing regions, there is a need to increase the resolution of vineyard irrigation. However, traditional methods of measuring plant water stress (i.e., pressure bomb, porometry, and soil moisture sensing) pose major complications when applied to individual vines. This manifests itself either in the inability of these methods to function at such a high resolution, or in the labor-intensive nature of taking single-vine measurements. Our work focuses on detecting the evapotranspiration rate of individual grapevines using a mass balance approach. Four individually potted vines were placed on load cells. Each vine was outfitted with four humidity sensors and a single anemometer. Two of the four vines were watered with 48 kg of water, while the remaining two vines were watered with 36kg of water only, every ten days to simulate variable water stress. From budbreak, the relative humidity, wind speed through the canopy, and mass of each potted vine were recorded continuously. Estimated values of evapotranspiration were calculated from a mass balance using the vine canopy as a control volume. These estimated values were compared to the absolute measurement of lost water mass recorded by the load cell. Additionally, the mass balance estimates were compared to continuous soil moisture measurements and porometric and pressure bomb data taken once and twice a day, respectively. This work is a continuation of a similar experiment conducted on a single vine, in which evapotranspiration rates calculated via mass balance showed agreement with the evapotranspiration rates from the load cell.

Funding Support: Cypress Semiconductor, Ernest Gallo Endowed Chair in Viticulture, and Till Guldimann

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Artificial Neural Network Model to Estimate Vine Water Status Using a Multispectral Camera aboard an UAV

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A field experiment was carried out to implement an artificial neural network (ANN) model for estimating the spatial variability of vine water potential of a drip-irrigated C*arménère* vineyard located in the Pencahue Valley, Maule Region, Chile (35°25´ LS; 71°44l LW; 90 m asl). For this study, a helicopter-based unmanned aerial vehicle (UAV) was equipped with a multispectral camera at very high resolution (6 cm × 6 cm), while a pressure chamber was used to measure the midday stem water potential (MSWP) at the time of the UAV overpass, near solar noon. A multilayer perceptron ANN type was used to develop a model, using as input nodes the spectral information from five wavelengths (550, 570, 670, 700, and 800 nm) and as output node, the MSWP. As a reference, correlations between MSWP and several spectral indices such as NDVI, MSR, GNDVI, PRI, and TCARI/OSAVI were developed in this study. There were significant linear correlations between MSWP and spectral indices NDVI, GNDVI, and MSR, with r^2 ranging between 0.32 and 0.35. The best correlation was observed between MSWP and MSR. The ANN model could predict MSWP with an r^2 value of 0.87 and a root mean square error of 0.12 MPa. Results demonstrated that multispectral cameras placed on an UAV could provide a good tool to evaluate the intra-vineyard spatial variability of vine water status.

Funding Support: Chilean National Science Foundation (CONICYT)

Deficit Irrigation Practices May Alter *Vitis vinifera* L. Resistance to Cold Injury: Empirical Evidence from the Field

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Deficit irrigation reduces seasonal carbohydrate supply and decreases starch concentrations in vegetative tissues. The specific role of starch metabolism in conferring tolerance to cold is still poorly understood. A decrease in cold tolerance after sequential years of deficit irrigation could limit sustainable productivity in arid, high-latitude production regions; however, the topic has received little research attention. In this study, multiple *Vitis vinifera* cultivars grown at two locations in replicated trial plots were visually rated for cold injury severity after six or more sequential years of deficit irrigation. Cold injury severity was estimated after budbreak by determining the percentage of total spur positions per vine without live shoots. The Merlot trial site was evaluated in spring 2007 after vines were exposed to sequential seasons of deficit irrigation at amounts that supplied 90, 70, or 35% of estimated vine water demand (ET_c). The second trial location contained 15 cultivars that were evaluated in spring 2014 and 2015 after sequential seasons of deficit irrigation at 70 or 35% ET_c. In the Merlot trial, the spring injury rating of vines irrigated at 35% ET_c was significantly greater (74%) than vines irrigated at 70 or 90% ET_c (35 and 6%, respectively). Greater injury was also observed in vines irrigated at 35 relative to 70%

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ET_c in four out of 15 cultivars in 2014 and in all cultivars in 2015. Results from this research suggest that sequential years of exposure to deficit amounts of water during the growing season may influence subsequent tolerance to cold. Understanding the underlying mechanisms by which water deficit may influence subsequent cold tolerance is required before deficit irrigation strategies can be customized to avoid undesirable influences on cold tolerance. The empirical observations reported here demonstrate that this topic warrants further investigation.

Funding Support: U.S. Department of Agriculture, Agricultural Research Service

Evaluation of Stomatal Conductance as an Irrigation Scheduling Tool in Grapevine

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Physiological detection methods for grapevine water status are used to schedule irrigation, but many of these processes are often laborious and time-intensive. Stomatal conductance to water vapor, estimated with a hand-held leaf porometer, provides rapid estimates of vine water status and is thus an ideal tool in irrigation scheduling. Stomatal conductance (mmol/m^2 s) and midday leaf water potential (MPa) of varieties Merlot and Chardonnay were recorded from bloom until fruit maturity. During this time, vines were subjected to varying levels of water stress. Midday leaf water potential and stomatal conductance differed between varieties and as the level of water stress increased, both stomatal conductance and midday leaf water potential decreased. A linear relationship between the two measurements existed, indicating that stomatal conductance responds in a similar manner to midday leaf water potential in these varieties and thus could be used as an irrigation scheduling tool. The abaxial density of stomata (stomata/mm^2) also differed between varieties and in Chardonnay, was influenced by degree of water stress. The density of stomata in combination with the phenomenon stomatal patchiness should be considered when using stomatal conductance to schedule irrigation. Stomatal patchiness, or non-uniform stomatal closure, leads to large fluctuations of stomatal conductance occurring in discrete patches across the leaf surface. Multiple readings per leaf may be required to accurately estimate stomatal conductance.

Funding Support: Northwest Center for Small Fruit Research

Vine Water Stress-Based Precision Irrigation Using a Wireless Network of Sensors and Controllers

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A management zone-based precision irrigation scheme that used specially developed leaf monitors to sense vine water status was implemented in an E & J Gallo Wineries vineyard located in Galt, CA, during the 2016 growing season. The experimental plot was divided into two management zones using unsupervised fuzzy classification based on soil and plant characteristics (texture, digital elevation,

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electrical conductivity, yield, and vegetation index). Twelve leaf monitors were deployed to measure vine water status. Each leaf monitor continuously sensed the temperature of a shaded leaf along with relevant environmental factors to develop an index that correlated with vine water status. Data from these leaf monitors were complemented by soil moisture sensors, pressure sensors, and flow meters. Latching solenoid valves were used to control water supply lines to manage irrigation. These sensors and controllers were connected to hubs with cellular modems to upload the data to the web so that the data can be accessed from computers or mobile devices from anywhere web access was available. The same system was also used to remotely actuate latching solenoid valves to manage irrigation. Throughout the season, precision irrigation management based on vine water status and an evapotranspiration (ET)-based grower irrigation were implemented. In the vine water stress-based precision irrigation scheme, attempts were made to maintain a leaf water potential (LWP) of -13 bar during the veraison period and -12 bar pre- and postveraison as per grower recommendations. Around 50% of ET requirements were needed during the veraison period to maintain desired LWP. Preliminary results show that overall vine water stress-based precision irrigation required 69% of ET-based grower irrigation amount. Yield and several quality attributes including Brix values were also collected and results will be included in the presentation.

Funding Support: E & J Gallo Wineries and California Department of Food and Agriculture

WEDNESDAY POSTER ABSTRACTS

Field Evaluation of GRN Rootstocks on the Growth and Productivity of Chardonnay Grapevines in the Salinas Valley

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Twelve rootstocks were evaluated for three years (2014 to 2016) in a Chardonnay vineyard near Soledad in the Salinas Valley of California. The site was previously planted with vines that were infested with phylloxera and root knot and ring nematodes and was not fumigated prior to replanting. The soil is a Chualar loam with an approximate rooting depth of 1 m. Vines were head-trained and cane-pruned to two canes and two spurs on a vertically shoot-positioned trellis. Vine spacing was 1.5 × 1.9 m (vine × row). The experimental design was a randomized complete block with eight replications of the 12 rootstocks using five vine plots. GRN1, GRN2, GRN3, GRN4, and GRN5 were compared to RS3, RS9, 1103P, 101-14M, SO4, O39-16, and St. George. Data collected included yield, components of yield, fruit composition, and growth components. Crop yield was significantly influenced by rootstock and the three-year averages ranged from 5.10 to 7.91 kg/vine. Yield separated into three groups, with St. George and 1103P being higher, and O39-16 and GRN1 lower. Higher cluster number was the factor most influencing crop yield. Higher-yielding rootstock selections also had higher cluster weight due to more berries per cluster. Pruning weights ranged from 0.34 to 0.85 kg/vine, with 1103P and St. George having the largest weights and O39-16 and GRN1, the smallest. Fruit composition was significantly affected by rootstock due to crop load and canopy differences.

Funding Support: No external funding

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Effects of Protective Covers on the Temperature of Young Grapevine Stems and Roots in Winter

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Young grapevines are particularly prone to winter cold damage because of their small size and exposure to lethally cold air that pools on the vineyard floor. The effects of protective cover treatments on the temperature of young grapevine stems and roots was evaluated over two winters. In winter 2015 to 2016, the treatments were: empty and sawdust-filled 2-L cardboard cartons, mounded sawdust, foam insulation wrap, and no protection (control). The same treatments plus a mounded soil treatment were applied in winter 2016 to 2017. Treatment effects on vine temperatures were influenced by snow cover and ambient temperatures in the previous week. Averaged over the treatment period, daily minimum temperatures of vine stems and roots were similar for the unprotected (control) and empty-carton treatments and were up to 1°C warmer for the sawdust-filled carton, up to 5°C warmer for mounded sawdust or soil, and up to 1°C cooler for the pipe-insulation wrap. Snow cover further increased the minimum temperature of vines under sawdust mounds in 2015 to 2016. Compared with no protection, foam insulation wrap increased the daytime maximum temperature of vine stems by up to 6°C, but decreased the nighttime minimum temperatures by up to 4°C. The mounded sawdust and soil treatments were the most effective at increasing nighttime minimum temperatures of vine stems; however, in spring 2016, a high mortality rate was observed for buds that had been buried in sawdust.

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

Assessing Variability in the Vineyard Through a Spatially Explicit Selective-Harvest Approach

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Vineyard variability is a limiting factor in commercial vineyards that leads to suboptimal management decisions. Ecophysiological variability was characterized in a commercial Cabernet Sauvignon vineyard in Sonoma County, California, using a combination of proximal sensing (NDVI, image analysis), terrain analysis by geostatistical modeling, and assessing primary and secondary metabolism of grapevines. The analysis was performed on a spatially dense grid, where primary metabolism was assessed every 10 days and proximal sensing of the vineyard was conducted monthly starting at E-L Stage 21. Primary metabolism variables were spatially interpolated and clustered in two homogeneous management zones according to similar physiological behavior. The two zones were well-separated according to 70% variance in plant water status that regressed well with the ecophysical characteristics of the study site. Secondary metabolism of berry skin and flavonol and anthocyanin composition were characterized with C_{18} reversed-phase HPLC. Statistical differences were observed between zones in primary and second-

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ary metabolism, but not in yield. The grape berry in the higher-water stress zone had lower Brix, but higher anthocyanin and flavonol content occurred in the zone with less water stress. The two zones were differentially harvested and vinified in triplicate. The wines showed statistical differences in chemical composition. The spatially dense sampling approach coupled with proximal sensing showed that plant water status is a reliable variable to discriminate between management zones because of its direct effect on secondary metabolism. Selective harvest can be a straightforward method to counteract variability in vineyards when ecophysical variability is too large to coalesce with variable rate management approaches.

Funding Support: USDA-SCRI

Grapevine Iron (Fe) Chlorosis: Root-Soil-Microbe Dynamics to Mobilize Fe in Alkaline Soils in Central Washington

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Chlorosis, or leaf yellowing, is a nutritional disorder induced by iron (Fe) deficiency that affects more than 50% of Concord vineyards in central Washington. When chlorosis is severe, it can reduce root biomass, shoot elongation, and yield. The exact cause of chlorosis is unknown, but several studies have shown that calcareous soils high in bicarbonate have a detrimental physiological effect on grapevines and that foliar Fe supplements typically resolve the issue within a season. When grapevines are exposed to high levels of bicarbonates and low levels of Fe, they produce greater quantities of the enzyme phosphoenolpyruvate carboxylase (PEPC), which leads to higher concentrations of citric and malic acids in the root tips to aid in Fe uptake. Acidification of the rhizosphere then reduces Fe^{3+} into the bioavailable Fe^{2+} form necessary for plant and microbial growth. However, in the alkaline soils of the central Washington grapegrowing region, bioavailable Fe may be rapidly complexed into orthophosphates like strengite within the rooting zone. We hypothesize that as grapevine chlorosis severity increases, greater concentrations of organic acids accumulate within the roots, resulting in rhizosphere microbial production of phosphatase to acquire bioavailable Fe. To test this hypothesis, we measured organic acid quantities in Concord grapevine roots and soil phosphatase activity via spectrometric methods in two different vineyards. The goal of this research was to determine the role of phosphorus in inhibiting the uptake of iron in strategy 1 plants to help reduce the economically detrimental effects of chlorosis.

Funding Support: Washington State University

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Salt Tolerance of Four Grape Rootstocks is Related to Root Architecture Traits

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This study investigated the effect of salinity stress on root growth of four grape rootstocks to understand how root traits relate to salt tolerance. Such traits are key to understanding salt tolerance, since roots adapt to environmental signals while acquiring nutrients and water. Greenhouse-grown 140Ru, O39-16, Ramsey, and Riparia Gloire were screened in 4-L pots with fritted clay at four salt concentrations: 0, 25, 50, and 75 mM NaCl. Plants were destructively harvested over three weekly intervals, with the first harvest occurring after one week of salt treatment. Roots were scanned and analyzed with the WinRHIZOTM image analysis system, which measured and classified root length, area, and volume. Leaves and roots were dried, ground, and analyzed for chloride content. O39- 16 accumulated the most chloride, followed by Ramsey, Riparia, and 140Ru. All rootstocks experienced a decline in root biomass, reduced root length, and increased specific root length as the Cl^- concentration increased. O39-16 had the largest differences between 0 and 75 mM NaCl, compared to other genotypes. At higher salt concentrations, the lateral to structural root ratio and chloride accumulation in petioles produced the same ranking and may serve as a useful way to screen for salt tolerance in rootstock breeding programs.

Funding Support: California Grape Rootstock Improvement Commission, California Grape Rootstock Research Foundation, American Vineyard Foundation, CDFA Improvement Advisory Board, California Table Grape Commission, Louise Rossi Endowed Chair in Viticulture, scholarship support for Cassandra from American Society for Enology and Viticulture

Under-Vine Cover Crops Mitigate Excessive Vine Vigor and Improve Soil Health in a Mature Cool Climate Vineyard

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In the northeastern United States, most vineyards have vegetation between rows while maintaining a weed-free strip directly beneath vines. Combined with the cool and wet climate of the region, the practice of reducing weed competition promotes excessive vine vigor, which often leads to increased management costs and poor grape quality. A three-year study investigating the impact of annual under-vine cover crops was conducted in a mature Cabernet franc vineyard located in Ovid, NY. Five under-vine ground covers including chicory, tillage radish, fescue, alfalfa, and native vegetation were established in early May to compare with an herbicide strip maintained with glyphosate. Predawn water potential was consistently reduced in plots maintained with tillage radish; however, vine pruning weight was reduced only in the relatively wet year of 2015. Primary shoot growth, lateral shoot growth, and pruning weight were reduced in chicory plots. Soil aggregate stability, organic matter content, and microbial respiration rate were enhanced by under-vine cover crops compared to glyphosate. The results indicated that chicory was an effective under-vine cover crop to mitigate mature grapevine.

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Fescue, alfalfa, and native vegetation could be considered potential substitutes for glyphosate spray to improve soil health with little direct effect on vines.

Funding Support: Toward Sustainability Foundation

Evaluation of Genetic Diversity in Wild *Vitis* from Northern and Central Mexico

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One of the centers of origin for *Vitis* species is North America. However, little is known about the wild *Vitis* species inhabiting Mexico. These species possess strong resistance to Pierce's disease and the dagger nematode, *Xiphinema index*, and may possess other valuable traits such as drought and salinity resistance. One of the first steps in using this germplasm is to characterize how Mexican species relate to those from the United States. DNA fingerprinting with molecular markers creates a rich genetic resource base to detect and examine genetic diversity in wild *Vitis* species. This study evaluated 47 wild *Vitis* accessions from northern and central Mexico acquired from a small germplasm collection in Toluca, Mexico. DNA was extracted and genotyped with a set of 22 nuclear simple sequence repeat (SSR) markers and the accessions were taxonomically described. The data are being investigated to clarify genetic diversity, population structure, and patterns of gene flow among these accessions. The study will provide new information about the wild grapevines of northern and central Mexico. It will also provide insight into previous studies of Mexican *Vitis* germplasm collected by H.P. Olmo and how these accessions can be used in breeding programs.

Funding Support: CDFA PD/GWSS Board, ASEV Student Fellowship, CONACYT-UC MEXUS Fellowship

Modeling Vegetative Vigor in Grapevine: Unraveling Implicated Mechanisms

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Mechanistic modelling provides an interesting tool to unravel complex biological phenomena. Our study describes the construction of a dynamic model for vigor, considering vigor as the interaction between the environment (soil and atmosphere) and plant physiology. To validate the model, 50 genotypes from the F1 progeny of Ramsey and Riparia GM, were evaluated. Plants with contrasting vigor were grown in a greenhouse during summer 2014 and 2015, pruned to a single shoot, and watered daily. Shoot growth rate (b), leaf area (LA), dry biomass, plant and root specific hydraulic conductance (K_h and L_{pr}), stomatal conductance (g_s), water potential (Ψ), and photosynthesis (A) were measured. Partitioning indices and specific leaf area (SLA) were calculated. The model includes an empirical fit of a hypothesized seasonal pattern of gibberellins (not measured *in situ*) based on

published seasonal patterns and reference values. Validation regression resulted in R^2 = 0.96 between predicted and observed data. MSPE = 0.0628, mean bias = 0.001, line bias = 0.0968, and random variation = 0.01. Simulating single-variable variation defined the individual effect of each variable on vigor determination and explained why "smaller genotypes have higher L_{pr}". It remains necessary to measure hormones to further refine the model. However, we propose that by measuring L_{pr} and SLA in young plants and including the hormone fit, the model can predict, with acceptable accuracy, the vigor of an adult plant.

Funding Support: Instituto Nacional de Tecnologia Agropecuaria, INTA - Viticulture and Enology, UC Davis

Vintage Advancement and Compression in Australia Due to Climate Change

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A shift in phenological development is the most conspicuous biological effect of recent warming, with advanced maturity of grapevines reported in Europe, North America, and Australia. Associated with the advancement in maturity are anecdotal reports of compression of the harvest period, with different varieties grown in the same region now reaching optimal maturity at similar dates and a narrower peak period over which a single variety matures. Given the capital-intensive nature of the wine industry (processing capacity is used at most for eight to 12 weeks per year), climatic trends that compress harvests have the potential to affect financial viability. This anecdotal evidence has been difficult to validate and quantify. Analysis of commercial maturity data from 1995 to 2015 suggested that vintage compression was driven by two facets. First, there was consistent reduction in the time interval between maturities of different cultivars grown in the same region. For example, in the McLaren Vale region, the range in dates between peak maturity of Chardonnay and later-maturing Cabernet Sauvignon was ~20 days in the mid 1990s and is now averaging closer to five days. Second, individual cultivars are also reaching maturity over a shorter period within one region. For example, Shiraz across the Barossa region reached maturity over a 30-day period in the mid 1990s and was reduced to a 15-day window by the mid 2010s. While this analysis does not allow separation of the effects of warming and management practices, there have not been step-changes in vineyard management during the study period. Regardless of the causes, the advancement in maturity and reduction in the duration of the window of peak maturity illustrate the challenges faced by wineries to process fruit over a shorter, more-intense period.

Funding Support: Wine Australia and the Australian Department of Agriculture and Water Resources, through the Filling the Research Gap program.

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Impacts of Two Viticultural Practices on Dynamic Spatiotemporal Hormone Accumulation in Pinot noir Berries

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Two common, yet expensive, practices used in viticulture are cluster thinning and cluster-zone leaf removal, which are intended to alter environmental conditions in hopes of causing the vine to respond in a favorable manner (e.g., improved fruit quality or disease management). Because the vine's response to environmental changes is principally hormone-mediated, we profiled the active forms, conjugates, and precursors of auxin and abscisic acid to understand the physiological effects of cluster-thinning and leaf removal on dynamic accumulation of these compounds in the berry. Clusters were thinned to 0.5 clusters/shoot in cluster-thinned vines and all cluster-zone leaves were removed in leaf-removal vines. Similarly-developing berries were identified at eight time points during the growing season and each individual berry's developmental profile was examined using multivariate analysis tools. The tissues of the berries were separated (seed, pulp, and skin) and pooled by their vine of origin in preparation for metabolite extraction. Phytohormones were extracted using a targeted method developed within our laboratory and analyzed using HPLC-MS/MS under selective reaction monitoring mode. Data collected over two growing seasons indicate a clear spatial and temporal distribution of the bioactive and conjugate forms of the analytes: the accumulation of most correlated with major developmental transitions. Analysis of the precursors, conjugates, and catabolites of the bioactive analytes revealed specific regulatory pathways used in grape berries. We quantified the effects of the two practices on the two hormones responsible for ripening initiation. The most notable effect was consistent reduction of ABA in seeds of leaf-removal vines, which correlated strongly with elevated accumulation of the conjugated form, ABA-GE The data suggest an impact of either light or temperature in modulating conversion of ABA into its glycosylated form.

Funding Support: American Vineyard Foundation

Comparing Nitrogen Fertilization in the Vineyard versus Supplementation in the Winery in Pinot noir and Chardonnay.

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The goal of this project was to understand how nitrogen (N) status and fertilization in the vineyard affects vine productivity and wine properties, and how N supplementation in the winery affects wine properties in a red and white cultivar. Four wines using four replicates from the vineyard are being evaluated in each variety: A) no fertilizer or winery addition; B) no fertilizer, +DAP in the winery; C) no fertilizer, +ORG-N in the winery; and D) N-fertilizer, no winery addition. N fertilizer increased vine N status in both varieties, but Chardonnay responded faster and showed greater change than Pinot noir. Must YAN levels increased in N-fertilized vines by 38% in Pinot noir (from 176 to 243 YAN) and by 90% in Chardonnay

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(from 99 to 189 YAN). Fertilizer use did not influence growth or yield of Pinot noir, nor growth of Chardonnay. Yield of Chardonnay in the +ORG-N in-winery (treatment C) was lower than in N-fertilized vines, but other treatments did not differ. Fruit solar exposure and vine water status were not altered by fertilizer use in either variety in 2016. After winery additions, the N-fertilized and winery-supplemented treatments (B, C, and D) had higher YAN than the control (A) in Pinot noir. In Chardonnay, the +DAP (B) and N-fertilized (D) musts had the highest YAN, +ORG-N (C) was intermediate, and Control (A) must was lowest. The Pinot noir musts from N-fertilized vines fermented one day faster ($p < 0.05$) than all other musts, even though YAN was similar in +DAP and +ORG-N musts. In Chardonnay, the control musts with the lowest YAN took ~2.5 more days to complete fermentation than all other treatments, but this was not significant based on endpoint alone ($p > 0.05$). The sensory analysis of 2016 wines will begin in summer 2017.

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Proximal Sensing and Stratified Sampling in Vineyards Provide Direction in Coalescing Vineyard Variability

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Variability in vineyards leads to suboptimal management and is a limiting factor in productivity. A commercial Cabernet Sauvignon vineyard was proximally sensed with electromagnetic induction for soil properties and active canopy sensors to derive a normalized difference vegetation index (NDVI) at modified E-L scale stage 11. The resulting sensing layers were subjected to geostatistical analysis and clustered to derive two management zones. Equal sample size stratified-random samples were allocated within each management zone to ground-truth the management zones. Exposed leaf area per meter of row was 20% greater in Zone 1 than in Zone 2. A completely randomized experiment was established within each zone, with untreated controls where grapevines in Zone 1 received leaf removal on the morning side and Zone 2 received 1.2× the applied water amount starting at the 3 to 5 mm berry growth stage. Grapevine primary metabolism was assessed every 10 days and proximal sensing of canopy reflectance was conducted monthly. Net carbon assimilation and transpiration were greater in Zone 2 until exposed leaf area coalesced at verasion, after which Zone 1 and Zone 2 performed similarly in primary metabolism. Berry skin flavonoids were characterized with C_{18} reversed-phase HPLC and proanthocyanidin subunits were analyzed by phloroglucinolysis followed by reversed-phase HPLC. There was no difference in yield per vine between Zone 1 and Zone 2. However, berry weight of Zone 2 was 11% greater than Zone 1 at harvest. Total skin anthocyanins or mean degree of proanthocyanidin polymerization of the two zones did not differ significantly when treatment application was directed by proximal sensing. The leaf area to fruit ratio of Zone 1 and Zone 2 also coalesced at the end of the season. The results provide proof-of-concept for expanding cultural practices directed by proximal sensing to management zones to decrease vineyard variability in large-scale vineyards.

Funding Support: USDA-SCRI

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Crop Load Management of Young Pinot gris in the San Joaquin Valley, California

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San Joaquin Valley (SJV) has 61% of Pinot gris acreage and the majority of Pinot gris crush volume (83%). Strong demand for this cultivar has encouraged growers to advance the time frame from planting to production to less than two years. This requires the permanent vine structures to be established in the first year, with the first crop expected in the second year. Cropping in the second year raises the risk of overcropping, with possible carryover effects in subsequent years. To identify the optimum crop level and economic threshold for young Pinot gris vines, a field trial was conducted in a commercial vineyard in Fresno, CA. Three levels of inflorescences were retained three weeks before bloom at 0, 1/2, and 1 inflorescence per shoot, and unthinned vines were used as a control. Fruit ripening was significantly advanced by thinning inflorescences prebloom. Yield was reduced by 100, 28, and 6% from the thinning. Yield compensation was associated with greater fruit set and berry mass, without rot issues. Total soluble solids at harvest was significantly lower in the control vines. Pruning weight was significantly affected, ranging from 0.46 to 1.18 kg/vine, and the Ravaz index (RI) ranged between 8.3 and 19.9. Based on our results in 2016, 4.2 kg/m row and 0.45 kg/m row are believed to be the yield level and pruning weight for young Pinot gris vines under the quadrilateral cordon spur-pruning system to achieve an RI of 10. This pilot study provides evidence that this yield level and canopy size can give winegrape growers the maximum yield without affecting fruit quality and the following year's crop. Yield and canopy data in 2017 and 2018 will continue to be collected to understand possible carryover effects.

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WEDNESDAY POSTER ABSTRACTS

Anthocyanins Contribute to Red Wine Flavor

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Diacetyl enhances the flavor of red wine and contributes to its fullness and depth. The diacetyl concentration in red wine is higher than that in white wine or other alcoholic beverages. Diacetyl is mainly produced by lactic acid bacteria during malolactic fermentation. However, some white wine varieties prepared with malolactic fermentation have low diacetyl concentrations. Yeast also produces diacetyl, which is a byproduct of branched chain amino acid (BCAA) biosynthesis. An intermediate metabolite of BCAA biosynthesis, 2-acetolactate, is oxidized non-enzymatically to diacetyl. We noted higher diacetyl concentrations in red wine than in white wine and examined the relationship between grape juice color and diacetyl concentration. These observations led to the hypothesis that some ingredients in red grape juice may influence yeast gene expression and contribute to higher diacetyl concentrations. We conducted a test fermentation by adding various fractions of red grape juice and measured the diacetyl concentration in the resulting wine. Malvidin-3-glucoside, an anthocyanin, was the main ingredient of a grape juice fraction that

showed the greatest diacetyl production. Subsequently, we performed another test fermentation after adding standard anthocyanin reagents (malvidin-3-glucoside, cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside). Although all the anthocyanin reagents promoted greater diacetyl production, the diacetyl concentration depended on the anthocyanin type. These results indicate that anthocyanins contribute to greater diacetyl concentrations in red wine. Next, we investigated the influence of anthocyanins on yeast gene expression. *BDH1* expression, important for the conversion from diacetyl to acetoin, was significantly down regulated by anthocyanin. These results support our hypothesis and suggest that a higher diacetyl concentration results from suppression of diacetyl metabolism by anthocyanins. To our knowledge, this is the first study showing that anthocyanins influence diacetyl production in wine by modulating yeast gene expression.

Funding Support: Sapporo Breweries, Ltd.

Phenolic Composition and Sensory Properties of Bonarda Wines from Mendoza Province (Argentina)

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Bonarda (*Vitis vinifera* L.), also known as Corbeau, is the second most planted variety after Malbec in Argentina. Bonarda is widely distributed in different regions of Argentina, with about 84% of its total acreage concentrated in Mendoza. Despite its economic importance, there is currently no information about the chemical and sensory composition of Bonarda wines grown under different agro-ecological conditions. This study characterizes the phenolic composition and sensory properties of Bonarda wines from different viticultural regions of Mendoza, Argentina. During the 2016 season, grapes from 16 viticultural locations (A to P) were harvested in triplicate and made into wine in 30 L food-grade plastic fermenters. Wines were analyzed for basic chemistry (titratable acidity, pH, and Brix) and for phenolic composition and color parameters. Additionally, a descriptive sensory analysis was performed with 10 panelists in four sessions, and eight attributes were established: color intensity, violet hue, fruity, eucalyptus, herbaceous, astringency, bitterness, and acidity. A principal component analysis including chemical and sensory variables indicated a clear effect of the agro-ecological conditions in each of these 16 locations on Bonarda wines. Luján de Cuyo (L) and Maipú (K) Bonarda wines showed higher phenolic potential, due to relatively higher concentrations of tannins and anthocyanins, and were described as more astringent and with higher color intensity than Bonarda wines from the other regions. Bonarda wines from Tupungato (M) and Lavalle (B) were characterized as being higher in perceived acidity, eucalyptus-like character, and fruity aromas and as having an intense violet hue. Bonarda wines from the northeastern and south regions of Mendoza showed lower levels of tannins and anthocyanins, less color, and a more pronounced herbaceous character. These preliminary results will be complemented by the study of individual phenolics (flavonoids and non-flavonoids), aroma compounds, and polysaccharides.

Funding Support: Instituto Nacional de Tecnología Agropecuaria (INTA) and Corporación Vitivinícola Argentina (COVIAR)

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Influence of Different Aging Tank Materials on Phenolic Composition of Malbec Wines

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Currently, the three most popular containers for wine aging are made of oak, concrete, and stainless steel. The oxygen permeability of these materials varies, potentially affecting wine phenolic chemistry. Empirical evidence suggests that concrete allows for slow microoxygenation (much like oak), while imparting no flavors of its own (unlike oak), but improving mouthfeel sensations (unlike stainless steel). To these potential advantages there is that of a relative higher thermal inertia when using concrete. Therefore, the aim of the present study was to evaluate the phenolic evolution of Malbec wines during aging in different tank materials. Grapes from Gualtallary in the Uco Valley of Mendoza, Argentina, were made into wine in 100 L tanks following a standard protocol. After malolactic fermentation, the wine obtained was divided into triplicate containers, including concrete eggs (2000 L), one-year-old French oak barrels with medium toast (225 L), and stainless steel tanks (100 L), for six months. Measurement of dissolved oxygen (by bioluminescence) and overall phenolic composition and color parameters were performed at selected sampling points during aging (June, September, and December). Concrete eggs allowed more dissolved oxygen in wines during the length of the study, with levels 2-fold and 3-fold higher than the one-year-old barrels and stainless steel tanks, respectively. This factor imparted a relative effect on phenolic composition, creating a tendency toward more anthocyanins, tannins, and polymeric pigments in wines aged in concrete eggs, without significant differences relative to one-year-old barrels. Additionally, the color parameters were also influenced, with wines aged in concrete eggs showing higher color intensity, h*, and b* and lower L* and a*, relative to the other two aging treatments. These preliminary results are being complemented with the study of individual phenolics and sensory evaluation of wines by a trained panel.

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Effects of Modified Saignée Techniques on the Chemical and Sensory Profile of Argentinian Bonarda and Malbec Wines

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The final wine tannin concentration is contingent not only upon the original tannin content in the grapes, but also on the amount of effectively extracted tannins, which may be regulated by tannin-cell wall interactions during winemaking. Anecdotal evidence shows that Bonarda wines (syn. Charbono) are inherently low

in tannins, suggesting lower tannin retention rates than Malbec, in which tannin retention is not problematic. Here we hypothesized that application of saignée (removing a portion of the must prior to alcoholic fermentation) may result in partial removal of cell wall material, thereby furthering tannin retention. We studied the individual and comparative effects of several modified saignée treatments on the chemical and sensory properties of Bonarda and Malbec wines. The grapes were processed using four different saignée techniques, targeted at modifying 16% of the total volume, including regular saignée (S), saignée paired with an equal volume of water addition (WS), saignée paired with an equal volume of must from Red Globe (a table grape cultivar; MS), and transfusion, which consisted of parallel fermentation of the removed juice and a posterior reincorporation of it postfermentation (TS). A control without saignée was also included, with all treatments performed in triplicate in 100 L fermentors. Only S and TS applied to Bonarda enhanced tannin concentrations above that of control wines, consistent with the cell wall hypothesis. For Malbec, the treatments had no positive effect on tannin concentration relative to control wines. A two-way ANOVA including chemical data identified significant ($p < 0.05$) treatment × cultivar interaction for wine tannin concentration, indicating a possible effect of cell wall material on tannin extraction in Bonarda grapes. From a sensory standpoint, S and TS Bonarda wines were perceived as significantly more astringent, while for Malbec, this was true for control and MS wines.

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Effect of Stem Additions and Microwave Extraction of Musts and Stems on Syrah, Merlot, and Cabernet Sauvignon Wines

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Microwave-assisted extraction (MW) at 1,200 Watts for 10 min to 45°C and MW of stems at 1,200 Watts for 10 min to 60°C were applied to musts and stems of Cabernet Sauvignon, Merlot, and Syrah grapes from the Central Coast of California. Grapes were made into wine in 60 L fermentors, applying the following treatments in triplicate (n = 3): control; MW of the must prior to fermentation (MW); 100% stem addition (100%-stems); and 100% stem addition + microwave of the stems prior to fermentation (MW-stems). Percentages of stems in the clusters were 6.5, 3.8, and 4.5% in Cabernet Sauvignon, Merlot, and Syrah, respectively. In Cabernet Sauvignon wines, addition of stems (with or without MW) lowered wine color saturation (C*) and the red component (a*), while MW did not enhance color: MW-stems significantly increased tannin content relative to 100%-stems and these two treatments also showed higher concentration of catechin. In Merlot, only 100%-stems lowered saturation and a*. While MW did not enhance color, addition of stems increased tannin content by 46% (MW-stems) and by 19% (100%-stems), and these two treatments also showed higher concentration of catechin. In Syrah, only MW-stems lowered wine color saturation (C*) and a*, while MW was not significantly different from the control in most color parameters. Addition of stems increased tannin content by 29% (MW-stems) and by 40% (100%-stem addition) and, like in Merlot and Cabernet Sauvignon, these

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two treatments also showed higher concentration of catechin. A two-way ANOVA with interactions including all treatments and varieties indicated that 100%-stems consistently reduced anthocyanins, but this effect did not manifest in MW-stems. Application of MW to musts prior to fermentation had no effect on anthocyanins, tannins, or wine color, but MW-stems enhanced tannin extraction and anthocyanin concentration relative to the 100%-stems.

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Impact of Hydrolysable and Condensed Tannins on Red Wine Color Stabilization

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Color is a primary factor in red wine quality. Red wine color is due to anthocyanins extracted from grape skins during wine fermentation. Then, during aging, anthocyanins are degraded while color still remains. This phenomenon is mainly due to the reaction of anthocyanins with other molecules, leading to formation of new, more stable pigmented compounds. The aim of our study was to determine the impact of enological tannins from different plant sources on red wine color stability during storage. Pinot noir and Cabernet Sauvignon wines were produced. Ten different tannins from acacia, tara, quebracho, oak, grape, pomegranate, and tea were tested. Those differed in their chemical nature, condensed or hydrolysable. After malolactic fermentation, they were added to the wine at three different concentrations: 150, 300, or 600 mg/L). Sampling was carried out every month over six months. Color characteristics were determined by spectrophotometry. Tannin concentrations, and small and large polymeric pigment content, were determined as described (Harbertson and Adams). Finally, anthocyanins were determined by HPLC. For both wines, the primary anthocyanin was malvidin-3-O-glucoside and its content decreased over time, as did the tannin concentration. Over the same time, the color characteristic remained quite stable. The evolution of wine pigments varied depending on the nature of the added tannins. Formation of small polymeric pigment was promoted by the tannin sources in Pinot noir wines, but less so in Cabernet Sauvignon wines. For both Pinot noir and Cabernet Sauvignon, small polymeric pigment were promoted by the addition of all tannins. Our experiments suggested that enological tannins had an impact on red color stability, but the effect depends on the nature of the tannin and the wine.

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Factors Affecting Acetaldehyde, Chromatic Characteristics, and Phenolics Evolution during the Oxidation of Red Wine

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Wine aging is an oxidative process regulated by wine initial composition, the presence of exogenous antioxidants, and total oxygen exposure. Although acetaldehyde is important for wine evolution, factors involved in its production, consumption,

and possible relationship with changes in phenolic compounds remain unclear. In a first experiment, the role of initial phenolic composition was examined with a forced oxidation simulating one year of barrel aging using wines with different anthocyanin/tannin ratios. In a second experiment, the same approach was used to evaluate the role of different enological tannin preparations (ellagi- gallo- and condensed tannins). In a third experiment, Cabernet Sauvignon wines previously treated with sulfur dioxide and glutathione and submitted to microoxygenation (Gambuti et al. 2015 AJEV), were bottled and analyzed after three years aging under controlled conditions. Wines with lower anthocyanin/tannin ratios produced less acetaldehyde and more polymeric pigments. Adding exogenous tannins has determined instead a greater production of acetaldehyde and an increase of the level of polymeric pigments in wine; ellagitannins were most efficient. In all wines analyzed, a successive dramatic consumption of acetaldehyde was observed. Acetaldehyde levels and color stability of wines after three years of aging were strongly affected by prebottling oxygen exposure: wines that were microoxygenated and less protected by sulfur dioxide and glutathione were richer in highly reactive compounds and stable polymeric pigments. In all experiments, for all variables considered, significant changes in tannins reactive to proteins were observed. These results confirm the role of acetaldehyde as a trigger compound for reactions that stabilize color and condense tannins.

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Influence of Polyphenol Adsorption to Grape Insoluble Polysaccharides on Polyphenol Extraction during Red Winemaking

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Tannins and anthocyanins are important components of red wine and their extraction mechanisms have been studied intensively. In general, red wines with a deep reddish color are considered better than those with light color. There are two types of grape tannin, originating from skin or from seed. Skin tannin has a more positive effect on wine taste than seed tannin, but the skin tannin concentration decreases during fermentation. Polyphenol from red grape skin can adsorb to insoluble polysaccharides and we hypothesize that this is one cause of polyphenol reduction during fermentation. We investigated the conditions under which tannin adsorbs to grape insoluble polysaccharides. Sample grape polysaccharides were separated and purified from skin, seed, and pulp and were subjected to model experiments with grape tannin. Grape tannin adsorbed to all parts of grape polysaccharide under the same conditions, but the adsorption rates were different. For example, in Muscat Bailey A, the adsorption rate was 5% in pulp, 30% in skin, and 45% in seed. Seed insoluble polysaccharide showed the highest affinity with grape tannin. The results indicate the importance of the conditions under which they react with each other.

Funding Support: JSPS KAKENHI

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Comparison of Tannin Addition Protocols and Their Effects on Extraction of Grape Phenolics during Red Wine Fermentation

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Extraction and retention of red wine phenolics, particularly anthocyanins and tannins, have historically been a primary concern for many red wine producers. Two competing protocols to facilitate the extraction and retention of phenolics by adding exogenous oenological tannin were compared for their relative efficacy. The first protocol involves adding exogenous tannin in a single full-dose at or immediately after destemming/crushing of the grapes. The second protocol, commonly referred to as the 'sacrificial' protocol, involves splitting the addition into two half-doses. The first half-dose, or sacrificial dose, is applied at or immediately after destemming/crushing, and the second half-dose is added a few days later. The specific mechanisms involved in the extraction dynamics are not elucidated here; however, phenolic profiling before and during fermentation and one full year after, using UV-Vis and AWRI tannin portal methodology, has shown a clear advantage to the single-dose protocol in extraction and retention of grape phenolics. Possible explanations are proposed and discussed.

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WEDNESDAY POSTER ABSTRACTS

Tannin Reacts with SO_2 during Aging, Yielding Newly Discovered Flavan-3-ol Sulfonates in Wine

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Tannins are large molecules that change during winemaking and bottle aging. Tannins trigger a tactile sensation of harshness in young red wines, while they appear to soften during aging. The changes in astringency are correlated with tannin structure alteration, such that large, condensed tannins (high DP proanthocyanidins) are degraded to shorter tannins. This reduces their binding strength with salivary proteins and is presumably the reason for reduced astringency. Under acid conditions, the interflavan bond of proanthocyanidins is labile, leading to bond cleavage and releasing a C-4 carbocation. The expected reaction is a rearrangement where the released C-4 carbocation reacts with a different flavan-3-ol subunit on the A-ring, restoring an interflavan bond. However, the electrophilic intermediate could be trapped by other nucleophiles such as thiols, which is the basis of the "thiolysis" method for analyzing the components of proanthocyanidin. Sulfur dioxide, widely used in winemaking, is also a nucleophile. However, to date, no products of SO_2 additions to proanthocyanidins have been reported in wine. In this study, flavan-3-ol sulfonates were detected in wine by LC-MS. Two of the major ones were isolated and investigated using Nuclear Magnetic Resonance Spectroscopy (NMR). They were identified as catechin-(4β)-sulfonate and gallocatechin-(4β)-sulfonate. The presence of these sulfonates and several other minor related compounds was demonstrated by LC-MS analysis. Furthermore, these compounds were measured in three wines aged with different SO_2 levels. The level of the flavan-3-ol sulfonates increased with higher levels of SO_2 added

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at bottling. Since sulfonate derivatives would have ionic properties, they are likely to have very different sensory properties than native tannins. Future studies will examine how those reactions could alter astringency and other sensory properties.

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Effect of Timing of Cluster Thinning on Pinot noir Grapes and Wines

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Cluster thinning is commonly practiced in Cabernet Sauvignon and Chardonnay, with little work done in Pinot noir from the Edna Valley in the Central Coast of California. In this three-year study, 50% crop reduction was applied at bloom (B), four weeks postbloom (B+4), eight weeks postbloom (B+8), and 12 weeks post-bloom (B+12) in Pinot noir grapes (clone 115) to evaluate the effect of the timing of cluster thinning on fruit and wine quality between treatments and against a control with no crop reduction. The vineyard experiment followed a complete randomized design (n = 5, ~100 vines/replicate) with three replicates made into wine. In 2016, cluster count was significantly reduced by 37% on average in the thinned treatments. Cluster weight was 43% lower ($p < 0.05$) in all four thinned treatments relative to the control, and, kg/vine decreased from 2.29 kg/vine in the unthinned control to 0.63 kg/vine in the B+8 treatment, giving an overall 66% yield reduction in the thinned treatments. There was no treatment effect on Brix and pH of the fruit at harvest, but berry weight decreased 18% in the B+8 treatment relative to the un-thinned control. Wine basic and phenolic chemistry showed marginal differences between treatments. Phenolics in wines (tannins, anthocyanins, and polymeric anthocyanins) showed no treatment effect; only catechin was marginally higher in B+4 and B+12 wines. Likewise, Cie-Lab color parameters (L*, C*, a*, b*, and H) and wine color (AU at 420+520 nm) showed no treatment effect. Overall, this preliminary study indicates no significant differences both inter-treatment and between treatments and the control in terms of grape and wine chemistry, in spite of a severe crop reduction in the thinned treatments, indicating a lack of effect of cluster thinning and the timing of cluster thinning.

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Chemical Effects of Cofermentation and Postfermentation Blending of Syrah with Selected *Rhône* White Varieties

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Cofermentation is an established practice within certain regions and areas of the wine industry and is speculated to improve wine color stability. The hypothesized reason attributed to Viognier cofermentation with red-skinned grape cultivars, most notably Syrah, is copigmentation (and thus color enhancement) resulting from a higher concentration of certain cofactors, primarily skin flavonols, which are potentially introduced by the Viognier grapes. In this study, a control wine (100% Syrah; Sy) and five cofermented Syrah blends consisting of additions

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by weight of 10% pressed solids of Marsanne (Mr), Roussanne (Rs), Viognier (Vg), Picpoul blanc (Pc), and Grenache blanc (Gb)) were made in triplicate 60 L fermentors (n = 3). In addition, prior to bottling of the finished wines, a portion of the 100% Syrah wine was blended with 10% (by volume) of Mr, Rs, Vg, Pc, and Gb wines (postfermentation blending). It was hypothesized that adding white grape solids would result in color enhancement and different phenolic profiles relative to 100% Syrah as opposed to postfermentation blending. At pressing, wines were analyzed by HPLC for phenolic content and by UV-Vis spectrometry and CIELab tri-stimulus colorimetry analysis to quantify wine color. Tannin and polymeric pigments were not significantly affected in any cofermentation treatment, but catechin and anthocyanin content were, with Sy-Vg and Sy-Mr showing higher anthocyanin content than other cofermentation treatments, but still lower than the Sy control. There was a statistically significant reduction in color amongst cofermented wines relative to the 100% Sy control, with Sy-Gb showing statistically larger reduction in color (chroma, a*) than other cofermentation treatments. Further color testing will be performed to evaluate color stability and sensory analysis of the wines will be performed to fully evaluate the effects of white cultivar cofermentation and postfermentation blending additions of Rhône white varieties on Syrah wines.

Funding Support: Wine and Viticulture Department - Cal Poly San Luis Obispo

WEDNESDAY POSTER ABSTRACTS

Comparison of Different Extraction Methods to Predict Anthocyanin Concentration and Color Characteristics of Red Wines

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Red wines ferment in contact with skins to extract polyphenols and anthocyanins that help build, establish, and stabilize color. The concentration and composition of anthocyanins varies among genera, species, and cultivars. For this study, eleven grapes representing *Vitis vinifera* (Cabernet Sauvignon, Merlot, Cabernet franc, Barbera, Syrah, Petite Sirah, and Mourvedre), *Vitis labrusca* (Concord), *Muscadinia rotundifolia* (Noble), and French-American hybrids (Marquette, Chambourcin) were selected. All cultivars were fermented on skins and color extraction was monitored daily. Each grape was also extracted using six different methods (microwave, and ultrasound-assisted, Glorie procedure, ITV standard, AWRI method, and solvent extraction of skins) and compared to color characteristics and anthocyanin profiles of the wines produced by fermentation. The extraction pattern varied significantly among cultivars included in this study. This is likely due to differences in extractability caused by variability in ripeness and structural differences among anthocyanins. Maximum color intensity was reached three to 10 days into fermentation, with considerable variation among cultivars. Postfermentation maceration, pressing, and sulfur dioxide addition lead to color loss up to 68% of the original maximum, with the highest loss in native American grapes and hybrid varieties. Color loss predominantly occurred in the red hue, followed by blue, with only minor changes in the brown part of the spectrum. Extraction procedures show a tendency to overestimate color in the finished wine, but are more accurate if compared to peak extraction levels during fermentation and are

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related to average berry and skin weight. Color loss and suitability of different extraction procedures to predict color characteristics of fermented wine strongly depend on the complexity of the anthocyanin spectrum. There is more variability for non-*vinifera* cultivars, since most extraction methods were developed for *V. vinfera*. However, ultrasound-assisted extraction shows potential for all cultivars because it is quick, reliable, and environmentally friendly.

Funding Support: N/A

Effect of pH and Ethanol on Tannin-Polysaccharide Interactions

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Tannins are considered to be the primary group of phenolic compounds responsible for astringency in red wines, with structure variation having an impact on perception. It has also been well documented that wine matrix can influence tannin perception. Ethanol, pH, and polysaccharides are known to affect the underlying process of astringency, or tannin-salivary protein interaction. To improve understanding of matrix consequences on tannin-polysaccharide-protein interactions, the pH and ethanol concentration of model wines was varied from 3.0 to 4.0 and 10 to 18% (v/v), respectively. Tannins and polysaccharides used for study were previously isolated from red wines. The formation of haze after tannin-polysaccharide interactions in different model wines was followed using spectrophotometry at 650 nm. The concentration of precipitable tannin after tannin-polysaccharide interactions in model wines and of mannan-red wine tannin interactions were characterized by a protein precipitation assay using bovine serum albumin. Our results showed that tannin-protein interaction varied as a function of pH, ethanol, and polysaccharide variation. Under the experimental conditions, precipitable tannins increased with pH and ethanol concentration. The effect of pH and ethanol concentration on mannan-tannin and on mannan-red wine tannin interaction was also investigated. Overall the results of this study indicate that tannin perception can be managed to an extent through pH, ethanol, and polysaccharide adjustment.

Funding Support: American Vineyard Foundation

Sequential Inoculation of Different Yeasts to Reduce Alcohol Concentrations of Red Wines

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Harvesting grapes at higher maturities requires modified winemaking procedures to reduce resultant alcohol levels without compromising sensory properties. Procedures employing non-*Saccharomyces* yeasts capable of respiring sugars without ethanol production have been proposed. However, excessive aeration that encourages these yeasts can impart off-flavors and increase volatile acidity. Non-*Saccharomyces* yeasts isolated from Washington vineyards were grown in high-sugar (310 g/L 1:1 mixture of glucose/fructose) Merlot grape juice, with or without 0.025 mL O_2/mL/min aeration, for six days prior to inoculation with *Saccharomyces cerevisiae*

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D254. While many strains reduced ethanol under aeration conditions compared to wines made with *Saccharomyces* alone, some produced unacceptable levels of acetic acid (>1.2 g/L). However, five species (*Metschnikowia chrysoperlae, Mt. pulcherrima, Meyerozyma guillermondii, Pichia kluyveri,* and *P. membranifaciens*) produced lower concentrations of alcohol and volatile acidity under aerobic conditions. In a second experiment, Merlot grape juice (24.3 Brix) was inoculated with these five species and additional strains obtained from other culture collections. Fermentations were capped with a gas-porous stopper to provide semi-aerobic conditions and after three days, inoculated with *S. cerevisiae* D254. Inoculation of *Mt. pulcherrima* yielded wines with significantly less ethanol, ~2.0% v/v less than wines inoculated with *S. cerevisiae* alone ($p < 0.05$). Additional isolates of *My. guillermondii* and *Mt. pulcherrima* reduced the alcohol concentration by 1.4 and 1.6% v/v, respectively. Additional trials were conducted in fall 2016 with Merlot grapes fermented in 300 L open-top fermenters and these strains. Ethanol, organic acids, and various odor/flavor compounds were quantified by HPLC and GC-FID.

Funding Support: Wine Advisory Committee

Impact of Honeybee Isolates of Lactobacillus kunkeei on Wine Fermentation

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Commonly known as "ferocious lactobacilli," *Lactobacillus kunkeei* represents a unique threat to wine quality due to its ability to rapidly produce high concentrations of acetic acid and induce a [*GAR*$^+$] prion state in *Saccharomyces* yeast. High levels of acetic acid can reduce yeast viability and wine quality, while a [*GAR*$^+$] prion state alters the ability of *Saccharomyces* to exclusively consume glucose and fructose sugars. Individually or in combination, these conditions can lead to stuck or sluggish fermentations. The source of *L. kunkeei* spoilage in wine is currently unknown. However, recent studies of the microbial ecology of honeybees (*Apis* sp.) has revealed *L. kunkeei* as the dominant microbial constituent of the gut microbiome of multiple species. Located in the foregut and crop of the honeybee, *L. kunkeei* acts synergistically with other microorganisms to enhance the innate immune system of the honeybee, inhibiting the growth of a variety of pathogenic and non-pathogenic organisms, some of which are associated with Colony Collapse Disorder. Given the abundance of *L. kunkeei* in the honeybee gut microbiome, we hypothesized that these domesticated and native pollinators may act as a vector for *L. kunkeei* in grape juice, resulting in wine spoilage and reduced fermentation efficiency. Fifteen strains of *L. kunkeei* were isolated from 28 honeybee samples and inoculated into small-scale fermentations of Chardonnay juice. Of the 15 *L. kunkeei* isolates, 13 grew well in 2016 Chardonnay juice. We hypothesize that *L. kunkeei* isolated from honeybees will cause stuck or slugglish fermentations, similar to the fermentation kinetics observed by our lab with *L. kunkeei* UCD26 strain in 2015 Chardonnay. Preliminary studies have shown that when compared to a control group, many strains of *L. kunkeei* isolated from honeybees possess inhibitory action towards yeast fermentation similar to that of the *L. kunkeei* UCD26 strain.

Funding Support: American Vineyard Foundation, Paul Monk Scholarship, Adolf L. & Richie C. Heck Research Fellowship, The Wine Spectator Scholarship

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Survival of *Brettanomyces bruxellensis* in Grape Pomace

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The ability of *Brettanomyces bruxellensis* to survive in infected grape pomace (GP) stored under different temperatures or placed in regional vineyards was examined. *B. bruxellensis* strains E1, F3, or I1a were inoculated into Merlot or Syrah GP samples (~75 g) which was placed into sterile milk-dilution bottles. Samples were initially incubated at 21°C for seven days for acclimation prior to storage at 21, 10, 0, or -18°C. All strains achieved populations >10^5 cfu/g by day seven of storage and remained ≥10^3 cfu/g at 10 or 21°C for the entire testing period. In comparison, populations declined to 10^2 cfu/g when stored at 0°C or achieved undetectable levels at -18°C within seven to 10 weeks. Additional GP was prepared from ongoing or completed fermentations-on-the-skins of Syrah grapes, with half sterilized by gamma irradiation (25 kGy). Samples (~100 g) were placed in sterilized bottles capped with 0.22 µm filters, inoculated with *B. bruxellensis* strain I1a, and placed in two vineyards located in the Columbia Valley and one in the Walla Walla American Viticultural Areas during fall of 2014 and 2015, respectively. Bottles were periodically removed to recover viable yeast cells using a *Brettanomyces* enhancement medium. Overall, yeast populations increased or decreased with seasonal temperatures but remained viable (~10^2 CFU/g) up to 100 weeks regardless of vineyard location. Pomace samples not treated with gamma-irradiation generally had lower populations than sterilized samples and yielded a wider variation in populations corresponding to seasons: populations detected during warm months could not be recovered during cold months. As *B. bruxellensis* can populate GP and survive at least 100 weeks, infected winery waste dispersed into vineyards may serve as reservoirs for further dispersal during subsequent grape harvests.

Funding Support: Washington Wine Advisory Committee

Impact of Storage Temperature and Ethanol on *Brettanomyces bruxellensis* Inoculated into Merlot Wine

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The potential for synergy between temperature and ethanol as a means to control infections by *Brettanomyces bruxellensis* in red wines was examined. Using a commercially-prepared Merlot wine, a four × five factorial experimental design was employed with storage temperature (12, 15, 18, or 21°C) and ethanol (12, 13, 14, 15, or 16% v/v) as variables. Culturabilities of two strains of *B. bruxellensis* isolated from Washington wines (I1a and F3) were monitored for 100 days before concentrations of volatile acidity, 4-ethylphenol (4-EP), and 4-ethylguaiacol (4-EG) were quantified. While growth of both strains was observed in 12 to 15% v/v ethanol, lag phase duration generally increased with a decrease in temperature. The two strains demonstrated similar growth patterns under various temperature × ethanol conditions except in those wines containing 15% v/v ethanol. At this concentration, F3 exhibited less growth and reduced concentrations of volatile

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acidity, 4-EP, or 4-EG at higher temperatures (18 and 21°C) than I1a. Strain F3 grew better at 18°C than at 21°C at this concentration of ethanol. Culturabilities of both strains quickly declined in wines containing 16% v/v ethanol. Wines in which *B. bruxellensis* reached >10^6 cfu/mL frequently contained concentrations of 4-EP and 4-EG in excess of 1290 and 155 µg/L, respectively, and above olfactory thresholds. Given significant interactions between temperature and ethanol, wines containing below13% v/v ethanol should be stored at ≤12°C to help limit spoilage by this yeast.

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Revealing Buried Treasures in Culture Collections

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Multiplex PCR in conjunction with microsatellite DNA analysis has been used to genetically type microbes over the last dozen years. It has been used successfully to type strains of *Saccharomyces cerevisiae* isolated from around the globe and there are several publicly available sequence comparison databases. We have used this technique over the last four years to type native California *Saccharomyces* strains collected from different isolated environments around central California. We initially used a database created in New Zealand that included over 80 commercially available yeasts and *Saccharomyces* isolated from New Zealand and other geographic areas around the world. The database also included over 30 sequenced strains from the *Saccharomyces* Genome Resequencing Project. We added to the database ~30 isolates from Chile and California and >50 additional commercial strains. This generated sequence from enough native isolate strains to determine a fingerprint for autochthonous *Saccharomyces* strains in California. We then evaluated the fingerprints of ~100 strains from both the UC Davis Viticulture and Enology Wine Yeast and Bacteria Collection and the UC Davis Food Science and Technology Phaff Yeast Collection. This was done to determine whether *S. cerevisiae* strains that have been in the collection for up to 79 years, with no information on their origins or characterized as natural isolates, had fingerprints consistent with recently isolated native yeasts or with commercial or laboratory yeast. We found that ~15% of the strains evaluated had fingerprints similar to the more recent set of autochthonous California isolates, with the oldest autochthonous accession entered into the collection in 1939. Another 20% were characterized as being possible natives or hybrids. The other 65% were closely related to commercial or laboratory research strains.

Funding Support: UC Davis Department of Viticulture and Enology, UC Davis College of Agriculture and Environmental Sciences

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Profiling the Yeast Microbiome in Pinot noir Vineyards and Spontaneous Fermentations at a British Columbian Winery

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Wine is produced by one of two methods: inoculated fermentation, during which a commercially-produced, single *Saccharomyces cerevisiae* yeast strain is used, or spontaneous fermentation, where yeast present on grape and/or winery surfaces carry out fermentation. Spontaneous fermentation is characterized by a diverse succession of yeast species, ending with one or multiple strains of *S. cerevisiae* dominating the fermentation. The resulting wines may be more organoleptically complex due to the participation of a wide range of yeast species. Yeasts participating in spontaneous fermentation are derived either from the vineyard or the winery itself. In the vineyard, yeast population composition is highly heterogeneous, differing between regions and even between adjacent vineyards. While the vineyard fungal microbiome has been profiled in wine regions worldwide, this has not yet been explored in Canada. Using amplicon sequencing of the intergenic transcribed spacer region, we examined the impact of winery environment on yeast population structure in spontaneous fermentations over two vintages by comparing yeast populations in aseptically fermented grapes from a British Columbian Pinot noir vineyard to populations in winery-conducted fermentations of grapes from the same vineyard. We also characterized the vineyard-associated yeast populations in two additional, geographically separate Pinot noir vineyards (1km radius) farmed by the same winery to assess whether distance drives yeast population structure. To examine yeast population dynamics during fermentation, we profiled yeast populations in 100 samples from various stages of fermentation immediately after crushing and at early, mid and late fermentation. Pinot noir yeast species populations will be compared to previously-described *S. cerevisiae* strain populations from the same fermentations. This research is the first to characterize vineyard-associated yeast populations in Canada and is the first step in a regional yeast population study to identify species with novel oenological applications.

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Identification of *Saccharomyces uvarum* from Spontaneously Fermented Pinot gris in British Columbia's Okanagan Valley

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Commercial wines are produced either by inoculating fermentations with known commercial strains of *Saccharomyces cerevisiae* or by spontaneous fermentations, which rely on yeast from vineyard and winery environments. Spontaneous fermentation is characterized by a diverse succession of yeast genera, species, and strains, with usually one or multiple strains of ethanol-tolerant *S. cerevisiae* domi-

nating the mid and final stages. One motivation for a winery to use a spontaneous fermentation is that the wine will contain a more complex sensory profile than an inoculated fermentation. This is likely due to the production of a wider range of metabolic by-products from a diverse yeast community. Spontaneous fermentations may also be used because resident yeasts partake in shaping the regional character (*terroir*) of the wine. In 2013 and 2014, we performed spontaneous fermentations of Pinot gris grapes from an Okanagan vineyard, where yeasts from different stages of fermentation were isolated. Surprisingly, we found that approximately half of the 1500 yeasts isolated from both vintages were strains of *S. uvarum*; as expected, the remainder of yeasts were *S. cerevisiae. S. uvarum* is more cryotolerant than *S. cerevisiae* and produces higher glycerol levels and lower levels of acetic acid; therefore, *S. uvarum* may be of commercial interest as an alternative yeast species for fermentation at lower temperatures. To characterize the *S. uvarum* strains isolated in our study and distinguish novel genotypes, we used a set of 11 microsatellite loci from previous studies to perform strain typing. We compared our *S. uvarum* isolates to *S. uvarum* strains previously identified in New Zealand. Our preliminary data suggests that we have isolated a population of *S. uvarum* that is indigenous to the Okanagan Valley of Canada and that it could potentially enhance the fermentation properties and sensory profiles of Okanagan white wines.

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Competition Between *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in Controlled Chardonnay Fermentations

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Although *Saccharomyces cerevisiae* is the most commonly identified yeast during alcoholic fermentation (AF) in winemaking, several species of non-*Saccharomyces* yeasts can compete with *S. cerevisiae* throughout AF and may end up dominating. These yeasts, which include strains of *Saccharomyces uvarum*, are often characterized as efficient fermenters with high tolerance to ethanol, allowing them to complete AF. *S. uvarum* is a cryotolerant yeast that produces high levels of glycerol, but low levels of acetic acid, and has been isolated as a dominant yeast from uninoculated fermentations in Canada, France, and New Zealand. We isolated strains of *S. uvarum* from spontaneously fermenting Chardonnay must at a commercial winery in Canada in 2015 and selected one strain to further assess its fermentative capabilities. To observe the effects of both initial inoculation conditions and fermentation temperature on the competitive abilities of the *S. uvarum* strain, fermentations of five different inoculation ratios were conducted at two temperatures (n = 3). To determine whether one yeast contributed more strongly to a wine's volatile profile than the other, post-fermentation samples were taken to analyze the fermentation-derived compounds of the resulting wines using HS-SPME-GC-MS. We found that *S. uvarum* was more competitive at a lower fermentation temperature, but was unable to outcompete the commercial *S. cerevisiae* strain

unless inoculated at a higher initial concentration. This research is of interest to both the scientific community and the winemaking community, because most *S. uvarum* strains identified in wineries are non-commercial, and may have potential for enhancing regional character in white wines, which are usually fermented at a lower temperature.

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Native Yeast Diversity in Two Washington Cabernet Sauvignon Vineyards Consists of Common and Unique Species

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Production and consumption of native wines, made without introduced microbes or amendments, has increased recently in the United States, including the Pacific Northwest. Aroma, flavor, and textural characteristics of native fermentations are driven by native yeasts associated with berries and winery environments. Native yeasts contribute to these characteristics throughout vinification, even in the presence of commercial *Saccharomyces cerevisiae*. To address a knowledge gap about populations of native yeasts in Washington State vineyards, we examined diversity on Cabernet Sauvignon berries from two vineyards located in different American Viticultural Areas in the 2015 harvest season, and tracked their populations in small-scale fermentations. We hypothesized that the berries from each vineyard would harbor yeast species reported in studies from other laboratories, but the species would vary between vineyards and some would be unique. Diversity was assessed using the ITS1 of the fungal internal transcribed spacer DNA and the D2 region of eukaryotic 26S rDNA. The ITS1 dataset yielded 1,467 fungal sequence similarity groups, called operational taxonomic groups (OTUs), 111 of which were annotated as yeast or yeast-like genera or species. The D2 sequence set yielded 286 fungal OTUs, 110 of which were yeast/yeast-like genera or species. The D2 analysis also revealed the extent to which grape berries harbored pollen, algae, mushroom spores, and other airborne microbiota. Of the ITS-based OTUs, *S. cerevisiae*, *Metschnikowia chrysoperlae*, *Hanseniaspora uvarum,* and *Aureobasidium pullulans* were most abundant in both vineyards, supporting our hypothesis of shared species. Five minor yeast species appeared to be unique to our sampled vineyards. *Metschnikowia* spp., *Hanseniaspora* spp., *Meyerozyma guilliermondii*, *Wickerhamomyces anomalus,* and *A. pullulans* persisted through early- or mid-stage fermentation. Amendment with 80 ppm SO_2 affected populations of specific yeasts and non-yeast fungi, but had no global effects. Our findings provide a basis for subsequent yeast diversity and wine quality studies.

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Effect of Strain-Dependent Variability in Yeast Cell Membrane Lipid Composition, Metabolic Activity, and Gene Regulation

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Different wine yeasts strains (*Saccharomyces cerevisiae*) vary in cell biomass formation under identical fermentation conditions in a juice or medium. Yeast strains that yield higher biomass complete sugar depletion more efficiently than lower-biomass yielding strains. To understand this variation in nutrient use efficiency (NUE), we used a multifaceted approach to assess metabolic and regulatory differences between yeast strains. Initially, we focused on four commercial wine yeast strains with varying NUE, Montrachet, Cote des Blanc, T306, and Uvaferm 43, using small-scale (400 mL) fermentations in synthetic MMM medium. The maximum cell concentration reached by these strains ranged from $OD_{600\ nm}$ 7.4 to 10.2. We analyzed intracellular and extracellular metabolites during fermentation using GC-MS and HPLC-RI, respectively. Complete analysis of the lipid profile of each strain was performed using QqQ LC-MS. In addition, we used a transcriptomic approach (RNA-Seq) to understand relevant transcriptional control mechanisms. Using partial least squares regression with metabolomic and lipidomic data, we observed 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 phosphotidylcholines and phosphotidylethanolamine in the yeast cell membrane correlated positively with higher biomass yields, higher concentrations of phosphotidylinositols have the opposite relation with biomass yield. To further confirm our results, we used series of mutants that overexpress key pathway enzymes. As a result of overexpression of FAS1 (fatty acid synthase), statistically significant difference is observed in NUE, biomass yield, and lipid profile.

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Isolation and Characterization of Lactic Acid Bacteria from Winery Wastes

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Wineries create large volumes of byproduct streams and waste, including grape pomace, wine lees, and grape stalk, which should be valorized or treated before disposal to prevent environmental pollution. Grape pomace is the solid residue left after juice extraction during winemaking and includes grape skins and seeds. Wine lees are the residue that forms at the bottom of recepticals containing wine, after fermentation or during storage. The aim of this work was to isolate and characterize the lactic acid bacteria (LAB) present in both pomace and lees to find LAB with optimal properties to be included in fermented food. Malbec grape pomace and wine lees samples were centrifuged and resuspended in MRS medium enriched with 5 g/L fructose and 3 g/L L-malic acid and adjusted to pH 5.0. After incubation at 30°C for 72 hr, samples were plated on MRS agar supplemented

with 1% cycloheximide (MRS-C). All plates were incubated aerobically at 30°C for 72 hr. Twenty colonies of each sample were selected and preliminarily identified by cell morphology, Gram stain, catalase and dextran production, and growth under different conditions, such as NaCl concentrations (2.5, 5.0, or 7.5%), pH (4.0, 7.0, or 9.0), and temperature (15, 30, or 45°C). Glucose consumption and D- or L-lactic acid isomer production were also determined. Of 40 isolated bacteria, 28 were LAB and produced both isomers of lactic acid from glucose. All LAB isolated from grape pomace and most from lees isolated were homofermentative; only six were heterofermentative. Our results showed the presence of LAB in winery wastes generally had high lactic acid production and optimal properties for inclusion in wine or other beverages, but further studies must be conducted.

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Enhancement of Antioxidant Activity in a Fermented Grape Juice Using Selected *Oenococcus oeni* Strains

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Fermented beverages, such as grape wines and fruit juice are associated with human health benefits due to the presence of high concentrations of phenolic compounds. In winemaking, the lactic acid bacterium *Oenoccocus oeni* performs malolactic fermentation (FML), a process that deacidifies wine by decarboxylating L-malic acid, contributes to microbial stability, and modifies the aroma profile and sensory impacts. This study investigated changes in the composition of sugars, organic acids, and phenolic compounds in a fermented grape juice by *O. oeni* strains in relation to antioxidant activity. *O. oeni* MS46 and MS9 were inoculated into juice prepared from Malbec grapes from the Cafayate region and bacterial growth, glucose, citric acid, malic acid consumption, and acetic and lactic acids production were evaluated. *O. oeni* MS46 was inoculated into wine elaborated by alcoholic fermentation to lead FML and the phenolic compounds content and antioxidant activity were determined and compared with commercial wines. In grape juice, both strains grew in 12 days, 5 to 10% of the glucose was consumed, and L-malic and citric acids were completely consumed. The consumption of glucose and citrate were accompanied by production of D-lactic acid and acetic acid; the molar yield indicated that acetic acid was mainly formed from citrate. *O. oeni* MS46 had the highest malolactic potential. Wine elaborated by *O. oeni* MS46 had the most antioxidant activity and this activity was related to the phenolic compound content. We selected *O. oeni* MS46, whose metabolic properties were tested in a natural environment and in wine for potential biotechnological application.

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Polyphenols from Cafayate Grape Pomace Display High Inhibitory Activity on Human Acetylcholinesterase

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Polyphenols are ubiquitous plant metabolites of increasing interest due to their benefits on human health. Inhibition of acetylcholinesterase activity is a very promising strategy because it may impact several pathologies such as myasthenia gravis, glaucoma, and Alzheimer's disease. This study analyzed the effect of phenolics-rich extracts from winery wastes on human acetylcholinesterase activity. Grape pomace of Torrontes and Malbec grape varieties was obtained from a Cafayate winery (Valles Calchaquíes, Argentina). From each sample, methanol and ethyl acetate extracts were obtained using maceration and soxhlet equipment. The total phenolic content and a preliminary phenolic profile in each waste sample were evaluated using Folin-Ciocalteu reagent and TLC assay. The acetylcholinesterase (AChE) activity of different extracts and pure phenolic compounds was determined by Ellman's colorimetric method and also using indoxylacetate as the chromogenic substrate. Human red blood cell acetylcholinesterase in its membrane-bound form was used as the target enzyme because this isoform was a good model of the more important isoforms associated with Alzheimer's and myasthenia gravis. Both grape pomace extracts noticeably inhibited AChE, but the red grape pomace was more effective at inhibiting enzyme activity. These findings suggest that waste material from the winemaking industry could be a promising source of anticholinesterase agents. These byproducts could have high added value, which will aid the regional economy.

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Development of New Yeast Strains for Lowering Ethanol and Increasing Glycerol Content of Wines

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As a result of climate change, rising sugar content in grape must and the concomitant increase in alcohol levels in wine are some of the main challenges currently affecting the winemaking industry. Among several solutions now under study, specialized wine yeasts isolated under different selective pressures hold promise for ameliorating this problem. Processes to produce such yeasts are comprised of intentional genetic modification based on selective cultivation without application of GMO-techniques. This objective was achieved by combining different mutagenesis techniques: the first and second mutagens differed and were chosen from the following: nucleotide-alkylating agent, nucleotide-deamination agent, and UV radiation. A first selection step was performed between the first and second mutagenesis steps, and a second selection step was performed after the second mutagenesis step, in which the mutants resulting from the preceding mutagenesis step were exposed to hypertonic medium and alcohol dehydrogenase inhibitor selection factors. Finally, these strains were subjected to RNA microarray tests which showed that genes of the HOG (High Osmolarity Glycerol) pathway were mainly affected by the mutagenesis experiments.

Funding Support: Erbsloeh Geisenheim AG

Bold type indicates presenting author

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Efficacy of Cleaning Chemistries on Typical Wine Microorganisms

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Cleanliness and sanitation are inherent parts of winemaking and are necessary to reduce microbial populations throughout the winery. If microbial populations are ineffectively controlled, detrimental effects can occur that impact wine quality, ageability, or wine appearance through problems such as cloudiness, off-odors, or gas production. This study assessed survival of wine microorganisms at contact times applicable to tank cleaning protocols. Past studies have been more general, looking at model organisms or pathogens not found in the wine environment. Isolates of common wine microbes were studied, including *Saccharomyces cerevisiae, Brettanomyces bruxellensis, Zygosaccharomyces bailii, Oenococcus oeni, Acetobacter pasteurianus, Pediococcus parvulus,* and *Lactobacillus casei*. The sensitivity of planktonic cells and biofilms to each agent was examined. These microbes were tested against commonly used chemicals, with an emphasis on "green" chemical alternatives, at concentrations relevant to wine tank cleaning regimes at different contact times, which ranged from 5 to 30 min. Chemicals tested included sodium and potassium hydroxide and citric acid, and less environmentally harmful chemicals, including hydrogen peroxide, peracetic acid, and potassium bisulfate. Analysis of planktonic cells showed that sodium and potassium hydroxide were most effective in significantly reducing microbial populations in all organisms studied, while the other chemicals tested were shown to have mixed efficacy.

Funding Support: Unfunded research

New Impedance Method Reduces the Enumeration Time of *Brettanomyces* Yeast Contamination in Red Wine

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Brettanomyces yeasts are a major spoilage concern for the wine industry worldwide, leading to undesirable sensory properties with subsequent economic losses. Red wines are most often afflicted with this type of spoilage. Currently, the industry employs time-consuming plate counting to detect and monitor *Brettanomyces bruxellensis* contamination in wines. *B. bruxellensis,* a fastidious, slow-growing organism, requires five days or more of incubation for visible growth on agar plates. Consequently, a need exists to develop a quicker, feasible method to detect and monitor this yeast. The aim of this study was therefore to determine the feasibility of using "direct" and "indirect" impedance methods to detect and enumerate *B. bruxellensis* in red wine. A reduction in incubation time from 120 hr down to 0.8 or 57.7 hr for samples with 4.2×10^7 or 1.8×10^2 cfu/mL, respectively, was achieved using the impedance methods. The "indirect" method proved more suitable than the "direct" method, offering faster detection times for a comparable level of accuracy. Overall, the "indirect" impedance method is a viable alternative to plate counting to detect and enumerate *Brettanomyces* contamination in the wine industry. Impedance technology decreases preparation and incubation times,

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thereby increasing throughput, reducing economic risk, and decreasing the chance of sample contamination.

Funding Support: University of Auckland

Impact of Nutrient Supplementation of Synthetic Grape Juice on Growth of Pediococcus spp. Post-Alcoholic Fermentation

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Commonly isolated from red wines, recent proliferations of *Pediococcus* spp. may be due to excessive supplementation of musts with nutrients not consumed during alcoholic fermentation. To assess the impact of excessive supplementation, synthetic grape juices were prepared containing low (60 mg N/L) or high (600 mg N/L) yeast assimilable nitrogen (YAN) and fermented by *Saccharomyces cerevisiae* D254. After 14 days, wines were sterile-filtered and inoculated with different strains of *P. parvulus*, *P. damnosus*, *P. inopinatus,* or *P. pentosaceus*. Concentrations of amino acids, titratable/volatile acidities, and ethanol were measured before and after inoculation using standardized methods. Of the six species/strains examined, only two (*P. parvulus* OW-1 and *P. damnosus* OW-2) exhibited logarithmic growth in the low-YAN wines, while none grew in high-YAN wines. Growth in the low-YAN wines suggests increased risks of *Pediococcus* spoilage in wines undergoing stuck alcoholic fermentations. However, all strains inoculated into the high-YAN treatments declined to undetectable populations shortly after inoculation. Wines that did not support bacterial growth were sterile-filtered and redistributed into 10 mL test tubes prior to supplementation with peptone, yeast extract, liver extract, Tween 80, cysteine, asparagine, manganese sulfate, biotin, or calcium pantothenate. After re-inoculation of *Pediococcus* spp., growth was monitored for an additional 24 days. The addition of yeast extract greatly improved growth of all six strains, while peptone improved growth for five of the six. Here, logarithmic growth was observed in low-YAN wines, where populations reached $>10^6$ cfu/mL, suggesting the presence of important growth factor(s) for *Pediococcus* in yeast extract and peptone. As further supplementation of high-YAN wines did not affect bacterial growth, the higher ethanol concentrations present may have affected the nutrient requirements of *Pediococcus*.

Funding Support: Northwest Center for Small Fruits Research

Evaluation of Native Vineyard Yeasts of Washington State for Biological Control of Botrytis Bunch Rot of Grape

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Botrytis bunch rot, caused by *Botrytis cinerea*, can cause significant economic losses to grapes, especially in wet, cool production regions, or during transport and storage. Public and grower concerns about chemical fungicides and the emergence of fungicide resistance can be alleviated in part by biocontrol strate-

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gies for *B. cinerea* and other grape pathogens. Our objectives were to evaluate native yeast strains for their ability to colonize Thompson Seedless berries and to inhibit *B. cinerea* growth *in vitro* and suppress rot symptoms on berries in the laboratory. Eleven yeast strains isolated from Washington vineyards were identified as *Aureobasidium pullulans* var. *pullulans, Candida saitoana, Curvibasidium pallidicorallinum, Metschnikowia chrysoperlae, Metschnikowia* aff. *pulcherrima, Meyerozyma guilliermondii*, *Saccharomyces cerevisiae,* and *Wickerhamomyces anomalus.* Populations densities of all strains grew from 200 cells to 6.0 to 6.6 log cells/mL after two days in berry tissue. *A. pullulans* P01A006 showed inhibitory effects against all *B. cinerea* isolates *in vitro*, while other yeast species inhibited one to three pathogenic isolates. The most reduction in symptom development *in vivo* occurred with *A. pullulans* P01A006, *Met. chrysoperlae* P34A004, P40A002, *Met. pulcherrima* P01A016, P01C004, *Mey. guilliermondii* P34D003, and *S. cerevisiae* HNN11516. Further studies will be conducted on the population dynamics and the contributions of indigenous yeasts in natural fermentations. Our findings indicate that native yeasts, that can contribute unique flavors to Washington wines, could provide effective *in planta* biocontrol against *B. cinerea*.

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In Vitro Antifungal Activity of Cat's Claw (*Dolichandra unguis-cati* L.) on Fungal Contaminants of Grapes

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Black rot is a disease caused by *Aspergillus* species, mainly *Aspergillus niger* and *A. carbonarius*. These fungi reduce the yield and quality of grapes. Wine made with rotted fruit has inferior chemical and sensory properties. Synthetic fungicides are commonly used to control *Aspergillus* species, but have several adverse effects that make their use problematic. This work evaluates the *in vitro* antifungal activity of plant parts of *Dolichandra unguis-cati* on growth of *Aspergillus niger* and *A. carbonarius*. Fractions were obtained from the plant leaves or stems by sequential extraction with dichloromethane (fCh_2Cl_2), ethyl acetate (fAcet), and methanol (fMeOH). They were assayed on conidial suspensions of *A. niger* and *A. carbonarius* by the microdilution method in YES semiliquid medium (2500 to 2.4 ppm). The concentration of the fractions required to inhibit 50% of fungal growth (IC_{50}) were calculated by probit analysis. Dot-blot bioautographies were carried out to establish the minimum inhibitory dose of fungal growth (MID). The composition of the most inhibitory fraction was preliminarily investigated by TLC and the antifungal constituents were detected by TLC bioautography. The fCh_2Cl_2 fraction from stems showed the most antifungal activity, with IC_{50} values of 1025 and 1066 ppm and DIM values of 625 and 1250 µg on *A. carbonarius*

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and *A. niger*, respectively. TLC bioautographies indicated an inhibitory activity associated with bands observed at Rf = 0.70 and Rf = 0.63 in the chromatograms observed under UV_{254nm}. Further research is needed to separate and identify these antifungal compounds.

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Effect of Wine Phenolic Compounds on Spoilage Lactic Acid Bacteria Isolated from Argentinean Wines

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The lactic acid bacteria (LAB), mainly belonging to the genera *Lactobacillus* and *Pediococcus,* can produce alterations in wine including the ability to synthesize biogenic amines and exopolysaccharide. The low molecular weight phenolic fractions (LMF) were obtained from Malbec (M), Cabernet Sauvignon (C), and Tannat (T) wines from Cafayate, Argentine. The effect of LMF on the viability of *L. hilgardii* 6F and X1B and *P. pentosaceus* 12p, isolated from Argentinean wines, were assayed at an equal concentration to wine (1X) and concentrated two (2X) or four (4X) times in synthetic wine medium (SWM), pH 4.5. Additionally, the effect of some pure wine phenolic compounds (PC), such as gallic acid (G), protocatechuic acid (P), *p*-coumaric (C) acid, cafeic acid (F), and catechin (H), were assayed individually at a final concentration of 400 mg/L. At different times, samples were collected to determine cell viability in MRS-agar medium and the effect on cell membrane integrity was evaluated by electron microscopy. The composition of LMF obtained by liquid-liquid extraction with ethyl acetate was determined by HPLC-DAD, showing a total concentration of phenolic compounds of 316.7, 229.3, and 311.1 mg/L for M, C, and T wines, respectively. In the presence of LMF at 1X and 2X concentration, all fractions diminished the growth rate without cellular damage. At 4X concentration, LMF produced microbial cellular death and damaged cell integrity. Among the individual PCs tested, the greatest inhibition of *L. hilgardii* 6F, *L. hilgardii* X1B, and *P. pentosaceus* 12p occurred with caffeic and *p*-coumaric acids, including damage to cell integrity. *P. pentosaceus* was most sensitive to all LMF and PCs assayed. These results represent a promising approach to wine preservation by using PCs against LAB spoilage and, consequently, eliminating or reducing the use of SO_2 in wineries.

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Selection of *Oenococcus oeni* Strains as Starter Cultures of Malolactic Fermentation in Wines from Tucuman, Argentina

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Malolactic fermentation is a desirable process carried out in red wines by lactic acid bacteria, mainly *Oenococcus oeni,* that lowers acidity, improves wine quality, and contributes to microbiological stability. Only bacteria well-adapted to adverse wine conditions can perform this process and are selected as possible candidates for starter cultures. Some technological properties of *O. oeni* isolates were evaluated, including adaptation to different unfavorable wine conditions and consumption of different carbon sources. Lactic acid bacteria were isolated from red wine from Colalao del Valle-Tucumán. The tolerance of 22 isolates identified as *O. oeni* to stressful wine conditions was assayed, determining growth as absorbance at 560 nm in MLO medium at different pH values (3.5, 3.7, and 4.0), ethanol concentrations (12 and 14%), and sodium metabisulfite concentrations (40, 80, and 160 mg/L). In addition, consumption of malic acid, glucose, and fructose were evaluated in MLO medium supplemented with L-malic acid, determining bacterial growth by counting cells spread on MLO agar. The *O. oeni* isolates designated RAM1 and RAM10 showed the greatest tolerance to stress conditions in wine. All isolates completely consumed the L-malic acid in the first 24 hr and consumed all glucose and fructose at 48 hr, reaching a population of 10^8 to 10^9 CFU/mL. The isolates RAM1 and RAM10 could be used as starter cultures due to their tolerance of stress conditions and ability to consume L-malic acid. This study establishes a basis to develop possible starter cultures well adapted to the ecological conditions of wines from Colalao del Valle, ensuring implantation in the natural environment.

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A Rapid and Inexpensive Spectrophotometric Method for Tyramine Detection

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Lactic acid bacteria (LAB) are the primary microorganisms responsible for biogenic amine synthesis in wine, organic compounds that affect human health and the quality of the final product. The presence of tyramine was evaluated using an inexpensive and rapid method implemented in our laboratory. The assay was a modification of the method used by Undenfriend and Cooper to detect tyrosine in human urine. The samples consisted of the supernatants obtained after growing 30 LAB isolates from Tucuman wines in decarboxilating culture medium. The bacteria were previously screened in agar medium as in Maijala and in a commercial decarboxilating Moeller broth medium to detect tyrosine decarboxylase. The bacteria were adapted in MRS-Tyrosine medium for five to 10 days and then in-

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oculated individually in the Moeller medium and incubated 96 hr at 28°C. Uninoculated Moeller medium was used as negative control. Several positive controls were performed by adding exact tyramine concentrations to uninoculated Moeller medium for calibration. All samples were filtered and clarified with polyvinylpyrrolidone or activated charcoal and then deproteinized with 10% trichloroacetic acid. The 20 selected LABs produced tyramine in different concentrations, with the highest concentrations in supernatant obtained from *Lactobacillus* sp. (23.24 mg/L). All experimental procedures were performed in triplicate. These results show that this assay is a fast, efficient and low-cost method to quantify tyramine in culture medium.

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Enological Attributes of *Hanseniaspora vineae* Yeast Contribute to Increased Chemical and Sensory Complexity

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During alcoholic fermentation, yeasts transform sugars present in the must primarily into ethanol, glycerol, and carbon dioxide, but also produce a set of compounds through secondary metabolism, including some aromatic and other compounds associated with wine color. Because these metabolites are important for the sensory quality of wines, it is important to understand and increase yeast diversity during fermentation to create unique wine profiles within a massive market. This study is an enological characterization of 11 strains of yeast identified as *Hanseniaspora vineae*, from which we have obtained positive contributions at the winery level. The yeast strains were evaluated to determine extracellular protease and β-glucosidase enzyme activities, fermentative capacity at low nitrogen levels, glycerol production, polysaccharide release, SO_2 and alcohol resistance, formation of aromatic compounds, and cell lysis sensitivity. Fermentation products were evaluated to determine sensory properties related to consumer preferences. The results obtained enabled verification of the variability among strains of this species for accumulation of some aroma compounds, also demonstrated through other analyzed parameters. However, some key attributes were consistent among the 11 strains, such as high protease and β-glucosidase enzymatic activity, low production of short chain fatty acids and ethyl esters, and high production of decanoic acid compared to *S. cerevisiae* strains. The most outstanding characteristic of the species *H. vineae* was the production of benzenoids and phenylpropanoid esters (concentrations above two orders of magnitude) and ~4-times greater rate of cell lysis than *S. cerevisiae* strains, a key characteristic for aging wines on the lies. This behavior was reflected in the sensory evaluation, where all the fermentations performed with *H. vineae* were considered superior.

Funding Support: CSIC Universidad de la Republica ANII Uruguay

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Aroma Precursors as Discriminant Factors in Rare Nonaromatic Red Grape Varieties

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This work describes the aromatic potential of minor and diverse *Vitis vinifera* varieties, particularly the glycosidic volatile compounds, how they are modified by extraction into wine, and how this analytical variable affects aroma composition. The *V. vinifera* varieties selected (Marselan, Arinarnoa, Ancellota, Caladoc, Egiodola, and Lacryma Christi) are cultivated in southern Uruguay. A procedure was developed and optimized to overcome analytical difficulties in isolating aromatic precursors from these nonaromatic red varieties. Arinarnoa was used to evaluate the precision and reproducibility of the method, and aroma compounds were analyzed by GC-MS. The proposed method reproducibly isolated the lower concentration compounds. We determined compounds such as C6 alcohols, norisoprenoids (3-hydroxy-β-damascone, 3-oxo-α-ionol, vomifoliol), volatile phenols (guaiacol, 4-vinyl-guaiacol), and vanillins. The characterized aromatic potential of several minor varieties, for the first time also including minor components, suggests that some of these could provide an excellent option for winemaking and commercial diversification strategies.

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Discrimination of Pinot noir wines from Six Districts in an AVA using Elemental Analysis

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Multi-element profiling of macro-, micro-, and trace elements in wine has been proposed as a means of establishing authenticity and typicity. Previous studies considered differences among countries and wine regions, but there is very limited information for wines made from grapes grown within the same wine region, let alone single-cultivar wines from within a wine district. Twenty-five Pinot noir wines from six districts within a California AVA were analyzed for 49 elements using a combination of inductively coupled plasma-mass spectrometry (ICP-MS) and microwave plasma atomic emission spectroscopy (MP-AES) instrumentation. All wines were from the 2016 vintage and were analyzed after ~six months of aging, with little or no barrel contact and not fined or treated for physical instabilities. Canonical variance mapping using the single elements and various ratios of elements revealed complete separation of the wines into five groups based on the district and tight clustering of the wines from each district within its group. The complete resolution is achieved with the first two factors, which together account for 87% of the total variance. The separation between district groups is three to five times the dimension of the district groups. The method has potential applica-

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tions in tracing authenticity and understanding the contributions of site to wine elemental composition.

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Effect of Optical Berry Sorting on Red Wine Quality

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The quality of fruit used for winemaking is extremely important for the quality of the resulting wine. To improve fruit quality, many smaller, high-end wineries employ laborers to hand sort individual berries after destemming to remove unwanted material such as raisins, diseased berries, unripe berries, and materials other than grapes (MOG). This can be costly, labor-intensive, and can slow down the process line. To reduce the cost of labor and increase throughput, many wineries are adopting optical sorting technology. The objective of this study was to investigate the effect of optical berry sorting by color on the quality of different red wine varieties. Grenache, Barbera, and Cabernet Sauvignon from the Robert Mondavi Institute and Tyree vineyards on the UC Davis campus were optical sorted and 11.9, 4.2, and 1.3% unripe berries and MOG were removed, respectively. Wines were made in triplicate from sorted and unsorted (control) fruit and in duplicate from rejected fruit/MOG. Wine aroma, taste, and mouthfeel were evaluated by descriptive analysis and results indicated differences among the wines. However, the degree depended on variety. Wines were also analyzed by the Adams-Harbertson assay for total anthocyanins, phenolics, and tannin and by HS-SPME-GC-MS for aroma profiling. Multivariate statistical analysis was used to investigate the relationship between wine composition and wine sensory attributes.

Funding Support: Napa Valley Vintners

Using the Flash Détente Technology to Effectively Improve Quality in Chilean Red Wines

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"Greenness" in wines can be defined as the perception of herbal and vegetal flavors, considered undesirable sensory attributes in red wines. These green characters are partially related to the presence of methoxypyrazines, C6 alcohols (*trans*- and *cis*-3-hexen-1-ol), and aldehydes (hexanal). Flash Détente (FD) is a technological process that uses a heat exchanger coupled to a vacuum flash evaporation chamber to rapidly heat grapes and remove volatile compounds, leaving a concentrated must and a condensate. Before fermentation, the must can be mixed with the condensate, which must be previously treated with activated carbon to remove undesired compounds. We evaluated the capacity of FD for reducing the "greenness" in Chilean Cabernet Sauvignon wines without affecting other quality aspects. Three treatments were addressed: red winemaking without

FD (control), red winemaking of FD-treated grapes (T1), and white winemaking of FD-treated grapes (T2). The pH, acidity, ethanol, total phenols, and color index of each resulting wine were measured using standard techniques. Concentrations of methoxypyrazine and C6 compounds were measured using GC-MS/MS. Sensory analysis evaluated the impact of treatments on wine quality and "greenness". T1 wine had a greater color index and more total phenols than the other treatments. No significant differences in hexanal were found among treatments. *Trans*- and *cis*-3-hexen-1-ol concentrations were significantly lower in T1 and T2 wines than in the control. Methoxypyrazine measurements are under statistical analysis. T2 wine had significantly lower levels of green bell pepper, cut green grass, and vegetal aromas than control wine. Also, it had greater levels of fruit aroma, juiciness, and global quality. FD is thus an effective prefermentation technology to produce wines with reduced "greenness."

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Potassium Polyaspartate, a New Compound for Tartrate Stabilization of Wine

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The presence of potassium bitartrate (KHT) crystals in a wine bottle, even if harmless, is a cause of wine depreciation by some consumers. The addition of compounds that inhibit postbottling potassium bitartrate crystallization remains one of the most easily applied and inexpensive approaches for winemakers to employ. Subtractive methods like cold stabilization or electrodialysis have some disadvantages associated with cost, energy consumption, and selectivity. On the other hand, the stabilizing effect of common additives like metatartaric acid or carboxymethylcellulose can be time-limited and sometimes negatively impacts red wine color or becomes ineffective. These limitations highlighted the need for a new solution that guarantees long-term stability while preserving wine quality. Potassium polyaspartic acid (KPA), a new and recently approved additive (OIV/OENO 543-2016), is a synthetic polyamide synthesized by the thermal polymerization of L-aspartic acid. The stabilizing property of KPA against bitartrate was investigated with an emphasis on dosage and efficiency. A full-factorial design (2^3) was used to estimate the thermal stability of KPA over time. Its effect on tartrate and color stability was compared to that of other additives commonly used in the wine industry. In addition to strongly inhibiting potassium bitartrate precipitation, KPA does not affect color stability and can be added close to final filtration without modifying wine filterability. Moreover, KPA remained effective in wines even after exposure to temperatures between 30 and 40°C for an extended period, although there was a loss of protection in white wines kept at higher temperatures up to a maximum of 50°C. The impact of KPA on the sensory characteristic of each wine was also determined by sensory analysis.

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Cost-Efficient Assessment of Washington Cabernet Sauvignon Wines

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Trustworthy wine quality assessment generally relies on the sight, nose, and palate of individual wine tasters of high reputation who have limited availability and capacity. Thus, finding a cost-efficient method to assess wine quality without relying on a human expert taster provides financial and logistical benefits. We used our own database of biochemical and organoleptic analysis of 60 different Washington Cabernet Sauvignon wines to investigate using artificial intelligence to effectively assess the overall quality of the wines based on laboratory analytical techniques. Three bottles of each wine were tested. The computer program was first trained by pairing 32 wine biochemical and other characteristics with the overall wine quality assessment of an expert taster, then optimized to pick the most cost-efficient subset of the 32 parameters that could satisfactorily assess overall wine quality. Significant prediction accuracy, in particular of very high- and low-end wines, was achieved with five measured/available characteristics. In order of importance, these are: co-pigmentation, age, region, red color, and total SO_2. To the best of our knowledge, this is the first study of this kind on Washington wines.

Funding Support: Central Washington University

The Control of Redox Potential during Wine Fermentations

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Redox potential is a common electrochemical measurement of the status of competing electron transfer reactions in biological or chemical media, including fermentations. The redox potential of juice is usually between +300 to +350 mV before the onset of yeast growth. The anaerobic fermentation of glucose to ethanol within yeast requires that cells maintain an internal redox potential of -290 mV for both NAD and NADH to exist. To maintain this internal value, yeast transport a number of components from and into the fermentation medium, causing the redox potential patterns to change significantly during fermentation. The redox condition of the juice during fermentation can determine whether or not certain reactions take place. One example is the electrochemical reduction of suspended elemental sulfur to hydrogen sulfide, which is sometimes formed during fermentation. Being able to control the redox potential during wine fermentation might mitigate this and have other effects on yeast metabolism and other redox reactions. This study developed a controller for the redox potential during fermentations using air and demonstrated its efficacy at the experimental scale (100 L fermentations) to control the potential at a set value. The control system consisted of a microcontroller that communicated with a commercial redox meter and probe. The controller would sample the meter every 15 min and turn on the

air if the probe reading was below the redox potential setpoint. The controller and its related air spargers could raise the redox potential by as much as 100 mV during mid-fermentation. Uncontrolled, inoculated fermentations were allowed to proceed normally without added air. Experiments have been performed successfully in triplicate white fermentations (Chardonnay) and red fermentations (Grenache and Mataro). Differences in the Brix curves were noted. These appear to be the first examples of wine fermentations with a controlled redox potential.

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Catechol-*o*-methyltransferase Modulates Anti-cancer Activity of Anthocyanins

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According to the American Cancer Society, colon cancer is the third most common type of cancer in the United States for both men and women. Consumption of a healthy diet including fruits and vegetables may help prevent this disease. Anthocyanins are a class of polyphenols that contribute to the color of foods such as strawberries, blueberries, and grapes. These compounds reduce the incidence of colon cancer, yet they are heavily metabolized in the body, limiting their anti-cancer activities. Specifically, anthocyanins are methylated by catechol-*o*-methyltransferase (COMT), transforming them into potentially less bioactive compounds. The anti-cancer effects of anthocyanins compared to their methylated metabolites remain to be fully understood. We compared the anti-proliferative activity of a Rubired grape anthocyanin extract as a single treatment and in combination with entacapone (COMT inhibitor) in two human colon cancer cell lines, HT-29 and Caco-2. The anti-proliferative effects of pure anthocyanin compounds, cyanidin-3-glucoside and delfinidin-3-glucoside, were also compared to combined treatments with entacapone. The combination treatments had greater growth inhibitory effects than the single treatments. This combination effect was more apparent in Caco-2 cells than HT-29 cells. The effect of entacapone on increasing the bioactivity of polyphenols may be due to inhibition of COMT, but also to increasing extracellular production of ROS (reactive oxygen species). Using the fox assay, we studied the levels of ROS (reactive oxygen species) in cell culture media treated with anthocyanins, with or without entacapone. The combination treatments resulted in synergistic production of ROS. COMT-catalyzed methylation could potentially weaken the anti-cancer activities of anthocyanins, and entacapone appears to enhance levels of anthocyanin-derived ROS. These findings suggest that combination treatment of anthocyanins and entacapone could be a viable natural treatment and preventative measure for colon cancer.

Funding Support: Cal State Bakersfield Start-UP funds.

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"Fingerprinting" of Washington Wines

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Washington State is the second-largest premium wine producer in the United States, harvesting over 200,000 tons of grapes per year and producing 16 million cases. With a total economic impact of ~$5 billion, wine fraud could have a significant negative effect on the industry. In an effort to reduce this risk, here we explore the use of advanced analytical techniques to "fingerprint" and build a database of WA wines that could classify each wine to its geographic origin. Focus is on the analysis of inorganic, chemically stable, and thus conservative tracers that remain constant in bottled wine, independent of aging. These techniques are analogous to those used by the European Union to mitigate against fraud and consist of the analysis of trace metals and isotopic compositions. Of particular importance in this context is the extraordinary capability of our recently acquired Inductively Coupled Plasma Triple Quadrupole Mass Spectrometer (ICP-QQQ), with which 66 elements can be quantified reliably at sub-parts per billion (ppb) levels, including relative isotopic abundances of some elements. Furthermore, with a different instrument, we will analyze the isotopic composition of water in wine, as it provides the geospatial origin of water. Statistical analyses will be used to establish significant similarities and differences among wines within the region and with selected wines from other parts of the world. Since measured parameters are controlled by vineyard soils and winery procedures, they should allow unique identification of region once a database is established.

Funding Support: Central Washington University

A Comparative Analysis of Additives for Enhancing White Wine Shelf Stability

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Riesling wine was treated with an array of different reagents used to promote increased shelf stability in wines not intended for long term cellaring. These reagents included independent and combined treatments of potassium metabisulfide, ascorbic acid, chitosan, potassium polyaspartate, gallic tannins, citric acid, and polyvinylimidazole polypyrrolidone (PVI). All treated and control wine samples were then subjected to an accelerated aging protocol. This included four weeks of weekly temperature cycling, with 12-hour periods of hot and cool temperatures, followed by a six-day holding period at room temperature. Samples were taken at zero, four, and eight weeks and analyzed for free and total sulfur dioxide, oxidative browning, iron and copper content by flame AA, low molecular weight phenolics and acetaldehyde by HPLC, and volatiles by GC-MS. Results indicated that those treat-

ments containing combinations of stabilizing reagents had mixed results in scaling linearly with their individual components in reducing oxidative products, hazes, and other indicators of instability. The combination of PVI and chitosan proved effective, while a combination of potassium metabisulfide, ascorbic acid, citric acid, and gallic tannins was less effective than the individual components. Overall, those treatments that removed metals from solution most effectively, such as PVI and chitosan, had an increased ability to stabilize wines by preventing increases in markers for advanced oxidation, like acetaldehyde and browning pigments.

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Fast Arsenic Speciation Analysis of Wines with LC-ICP-QQQ

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Arsenic (As) occurs naturally in the environment and is consequently found in food and beverages such as wine. Arsenic exists in multiple forms, but not all species have the same toxicity. Due to the potential health threat of some species, it is important to measure individual species and not just the total As concentration. Regulations have been proposed for the more toxic, inorganic As species (As^{V} and As^{III}) in other food products. Traditionally, the inorganic As values were achieved by measuring individual species using ion exchange high pressure liquid chromatography coupled to a triple quadrupole inductively coupled plasma - mass spectrometer (HPLC-ICP-QQQ) and then the two inorganic forms were added together. Our method also used HPLC-ICP-QQQ, but instead of analyzing the inorganic As species separately, As^{III} was intentionally oxidized to As^{V} with hydrogen peroxide prior to analysis. This allowed all inorganic As to be expressed as As^{V}. By converting the inorganic species, this method separated monomethylarsonic acid and dimethylarsinic acid from As^{V} in 2 min, 10 times faster than the current Food and Drug Administration methods for speciation of As. Furthermore, by reacting samples with O_2 in the ICP-QQQ, there was decreased spectral interferences and increased sensitivity. Validation results from two participating laboratories are presented to demonstrate the new method's accuracy and reproducibility in wine matrices.

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Enology and Viticulture – CONTINUED

Rapid Detection of Alcohol Content in Wine by Surface Tension

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Quantification of alcohol by volume (ABV) in alcoholic beverages is required for commercial sales and is of significant interest for home winemakers. However, the process usually requires large sample volumes, multiple measurements over time, skilled personnel, and/or expensive instruments. Despite the wide variety of compounds in wine (including large differences in Brix between dry and dessert wines), there is a strong correlation between alcohol content and surface tension (R^2 = 0.974). Therefore, we report an alternative, instrument-free method of alcohol quantification in wine based on surface tension. A droplet of wine (3 µL) is placed onto the mesh sensor and will remain "beaded up" (>5 min) if the alcohol content is below the rated ABV, but will rapidly (<30 sec) wet into the mesh, causing a color change, if the alcohol content is above the rated ABV. These meshes can resolve down to 0.5% ABV differences in wine, ranging from 12.5 to 16% ABV. The sensors are composed of two layers: a top "responsive" layer and a bottom "indicator" layer. Both layers are fabricated by electrospinning, a well-controlled, robust commercialized manufacturing process. The lower indicator layer, composed of 5% bromocresol purple, 5% poly(glycerol-*co*-ε-caprolactone), and 90% poly(caprolactone), facilitates wetting and the color change, while the upper responsive layer, composed of 3 to 50% poly(glycerol monostearate-*co*-ε-caprolactone), selectively allows wetting below a specified surface tension and rated ABV. This facile, affordable, and portable method of accurately determining up to 0.5% differences in ABV within 1 min is sufficient to meet United States labeling standards using only µL samples.

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Comparison of Bourbon and Scotch Whiskies Using Phenolic Hydrophobicity and UHPLC-QTOF/MS

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The hydrophobicity of a set of 15 bourbon whiskeys and 16 Scotch whisky samples was determined by octanol-water partitioning modified from published methods (Mueller-Harvey et al. 2007, Zanchi et al. 2009). Whiskey, water, and octanol were combined in a 15 mL centrifuge tube and mixed for 30 sec. The tubes were centrifuged to separate the layers and an aliquot of each layer was analyzed via HPLC. HPLC analysis was carried out on an Agilent 1200 HPLC system (Santa Clara, CA). Detection used a diode array detector at 280 nm; total peak area was determined for each phase. The octanol/water partition coeffi-

cient (K_{ow}) was calculated for each whiskey by dividing the total peak area of the octanol phase by the peak area of the water phase. The partition coefficients for the bourbon whiskeys spanned a narrow range between 1.3 and 1.6. The partition coefficients for Scotch whiskies were more variable, but generally lower than for the bourbon whiskeys. The composition of the water and octanol phases was also characterized using UHPLC/QTOF-MS. This analysis was conducted on an Agilent 1290 UHPLC/6545 QTOF-MS system using electrospray ionization (ESI) in negative mode. The MS data was analyzed using the Agilent MassHunter and Mass Profiler Professional software. The water phase contained lignan glycosides, while the octanol phase was characterized by lipids, phenylpropanoids, lignans, and triterpenoids. Triterpenoid glycosides were present in both phases. Using principal component analysis, bourbon and Scotch whisky samples were readily differentiated based on the composition of their octanol phases. Both the partition coefficients and the composition of the two phases can be related to the use of Sherry butts versus American oak casks and the number of fills for American casks.

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Profile and Content of Fatty Acid in New and Used Bourbon Whiskey Barrels

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Barrel maturation imparts volatiles into the distillate; these volatile compounds arise from different components of the wood breaking down over time. These breakdown products are influenced by barrel modification, weathering, kiln drying, and thermal degradation. Fatty acids are a minor element in wood and are found in whiskey, but are generally filtered out before sale. This element of wood-distillate interaction has not previously been studied. It remains unclear whether fatty acid content and profile change during whiskey maturation and barrel modification. In this study, we examined the total content of fatty acids by preforming a soxlet extraction on barrel wood with petroleum ether and measuring the total weight change after completion. Fatty acid profiles and abundance values were established using GC FID. The extracted fatty composition showed changes from both barrel modification and from maturation of whisky. We continued surveying the fatty acid profile to include various fractions throughout the barrel stave and found that content and compositional shifts occur throughout the barrel staves.

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Enology and Viticulture – CONTINUED

Surveying the Composition of Phenolic Compounds in Bourbon Barrel Staves

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Bourbon whiskey is aged in new American white oak barrels that are charred. Charring of barrels produces a unique environment that the distillate interacts with from two to 23 years. The overarching hypothesis of this study is that the process of barrel charring disrupts the naturally occurring biopolymers in the wood and produces breakdown products that are desirable flavors. Here, we focused on the phenolic fraction of compounds found in the wood. This fraction consists of lignin, hydrolyzable tannin, and breakdown products formed during charring from cellulose and hemicellulose. The production of these breakdown products is highly variable in oak products. In this study, we surveyed lignin, tannin, total extractable phenolic compounds, and phenolic compounds in situ and how these values change from barrel charring and bourbon maturation. We furthered these analyses by looking at the interior, middle, and exterior subsections of new and used barrel staves. Subsections of charred barrel staves indicate that phenolic and tannin content is higher in the interior of the barrel stave and decreases during maturation, while the lignin content was unchanged. Methanol extraction of the barrel interior subsection followed by GC-MS showed that compound profile and abundance shifts occur through the depth of the stave. To complete this analysis, the surface fraction of the charred interior was subjected to PYR-GC-MS, revealing even with the variation from stave to stave, some trends were found revealing a fingerprint of a flavor profile for each barrel surveyed.

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