# Technical Abstracts

70th NATIONAL CONFERENCE



Napa Valley Marriott Hotel Napa, California USA



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AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

### **Technical Abstracts**

#### **Oral Presentation Abstracts**

#### Wednesday, June 19

Enology—Phenolic Extraction	
Viticulture—Impact of Red Blotch on Grape and Wine Composition	
Enology—Microbiology of Wine	
Viticulture—Managing Pests and Weeds	
Enology—Wine Chemistry: Oxidations and Aging	
Viticulture—Fruit Composition and Yield	61-63
Thursday, June 20	
Enology—Wine Macromolecules	64
Viticulture—Crop Load Management	65-66
Enology—Wine Stability	
Viticulture—Breeding and Rootstocks	68-70
Enology–Vineyard and Winery Impacts on Wine Sensory	
Viticulture—Irrigation	

### **Poster Presentation Abstracts**

#### Wednesday, June 19 & Thursday, June 20

Enology & Viticulture	32
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**NOTE:** Pages 1-45 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.

### **Technical Abstracts (Continued)**



### Alphabetical Listing of All Authors with Submitted Abstracts

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gu , D an lei ura n, J d, J hs, X nai cíne k, C ds fiel ura yer iok
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Jin
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ifi,
ner
ora

Jaeger, J131
Jarugula, S119
Jing, D78
Kaplan, J
Keller, M75
Kenney, P
Kovalenko, Y
Kurtural K 112
Levin A 53
Litwin J 53
Lovd J 72.81
Lund K 113
Lyons S 99
Lyu X 77.82
Maimaitiviming M 113
Martípoz-Lüschor I 109
Mary C
Marx, C
Mawdsley, P
Mayneid, S129, 130
McGourty, G102, 103
Medina Plaza, C49
Moreira, L116
Morgan, S95
Nemoto, M128
Nguyen, T79
Nishioka, K107
Noestheden, M110
Norton, E50
Ogawa, M94
Okuda, T 89
Olejar, K59
Perry, D78
Peterson, A59
Phan, Q95
Pigao, K94
Postiglione, D87
Postoronca, M77
Preto, C 51
Ramagli, M127
Reiter, T 55
Rex, F97
Riaz, S117, 118
Rice, S80, 81
Ring, L64
Rumbaugh, A 51
Ruppel, M70
Seguinot, P102
Serra, I
Shackel K 126
JIIdCKEI, K
Sharifi, M107
Sharifi, M
Shacker, K

Svyantek, A	68
Tanabe, C	61, 80
Threlfall, R	109
Tian, T	75
Tondini, F	105
Trela, B	104
Usher, K	106
VanderWeide, J	65, 117
Vega-Osorno, A	
Villalpando, I	
Wang, X	116
Weber, F	68
Wegmann-Herr, P	
Yan, G	96
Yu, R	126
Zhuang, S	.70, 124, 125

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

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### **Enology**—Phenolic Extraction Session

# Elucidating and Modeling Proanthocyanidin Adsorption and Desorption Phenomena in Model Wine Systems

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The kinetics and quantitative and qualitative effects of grape proanthocyanidin (PA) adsorption and desorption with grape skin-derived cell wall material (CWM) were investigated under various temperature and ethanol conditions. Studied conditions ranged from 15 to  $35^{\circ}$ C and 0 to 15% ethanol (v/v) in model wine. Adsorption progress curves for each condition were constructed by monitoring the decrease in concentration of PA solutions after exposure to CWM over 720 min. Adsorption isotherms were constructed for each condition, and a Langmuir Equation model was applied to each via regression analysis. Desorption experiments were performed by allowing PA and CWM to equilibrate at 15°C, incrementally increasing temperature to 35°C, and monitoring the increase in PA concentration during this temperature gradient. For both adsorption and desorption experiments, compositional changes in the PA solutions after exposure to CWM were determined using phloroglucinolysis and gel permeation chromatography (GPC). Results show a negative correlation between PA adsorption and increases in both temperature and ethanol concentration. Langmuir models applied to each adsorption condition show acceptable fits. Adsorption progress curves suggest that both temperature and ethanol effect the equilibration rate of the adsorption reactions. Additionally, equilibration rates for lower temperature and ethanol concentration conditions were much longer than previously reported, the longest taking between 480 and 720 min. Desorption experiments revealed temperature-dependent, reversible PA-CWM adsorption reactions. Data suggest that key ethanol concentrations exist between 0 to 7.5% and 12 to 15% that significantly impact the extent of desorption. In both adsorption and desorption experiments, phloroglucinolysis results showed no discernable correlation between temperature or ethanol concentration and % galloylation or % gallo units. Consistent with previous adsorption studies, results from GPC analysis showed a preferential adsorption of larger-molecular weight PA over smaller-molecular weight PA under all conditions. Additionally, findings suggest that both temperature and ethanol concentration impact the percent reduction in molar mass of the PA solution.

Funding Support: E&J Gallo

#### Impact of Anthocyanins on Skin Tannin Extractability

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Previous work conducted in 2015 and 2016 suggested anthocyanins were modifying tannin extraction in benchtop experiments. To measure the impact anthocyanins have on tannin extraction, a study was implemented in 2018 using Sauvignon blanc. Berries were sampled at five time points, starting at lag phase and ending at commercial harvest. Density flotation was conducted to ensure similar maturity in the berries extracted. After flotation, the skin tissue was separated from pulp and seed by hand. For wine-like extraction, skins were shaken in a 16% v/v ethanol solution, buffered with citric acid at pH 3.3, for 72 hrs. The control contained no anthocyanins, while the treatments had 300 mg/L or 1000 mg/L anthocyanins added into the 16% v/v ethanol extraction. Upon completion of wine-like extraction, the skins were frozen, ground to a powder with

#### Bold type indicates presenting author



#### Enology—Phenolic Extraction Session—CONTINUED

liquid nitrogen, and then extracted with 66% acetone for complete extraction. Extracts were analyzed for anthocyanin and tannin concentrations, tannin pigmentation, and subunit composition. Extractability was measured as the fraction of tannin extracted in the wine-like extraction compared to the total tannin obtained from both extractions. The results suggest that anthocyanins impacted the extraction of tannin into a mild alcoholic solution. These results suggest that anthocyanins may be altering extraction of tannins into red wine during the maceration process. Winemaking experiments are underway to evaluate this possibility.

Funding Support: American Vineyard Foundation

#### Vintage Effect on Skin Cell Wall Composition and Phenolic Extractability of Cabernet Sauvignon from Different Regions

**Cristina Medina Plaza**, Nick Dokoozlian, Ravi Ponangi, Tom Blair, David Block, and Anita Oberholster\* \*University of California, Davis, 595 Hilgard Ln, Davis, CA 95616 (aoberholster@ucdavis.edu)

The phenolic component of red wine is responsible for important elements of the flavor, mouthfeel, and quality of the finished wine. While the origins of the phenolics in a finished red wine are well known, the extraction of these compounds from grape skins and seeds during red wine fermentation is poorly understood. The skin cell walls of berries play a very important role during the winemaking process, as they can form a barrier to the release of important components. In this work, phenolic extractability of Cabernet Sauvignon from two regions within California (Sonoma and Central Coast) was investigated over two different vintages (2017 and 2018). The study included the analysis of phenolic berry composition, their potential extractability during winemaking practices, as well as the skin cell wall composition (CW). Differences in grape phenolic composition between regions and sites were found for all phenolics studied. Using multivariate analysis, clear separation between vintages and regions was found, suggesting that phenolic composition and extractability are directly impacted by region and vintage. No correlation was found between berry composition and the amount extracted for any of the phenolics analyzed, although in general Central Coast grapes contained fewer phenolics and had lower extractability. CW composition was determined in an effort to relate its differences among sites with the differences found in phenolic extractability. CW composition differences were found between regions for all parameters studied, especially for uronic acid, protein, and lignin. Uronic acid content had the most impact on phenolic extractability for all samples analyzed, independent of region and vintage, showing that the amount and solubility of the pectin are key in the extractability process.

Funding Support: E&J Gallo Winery

#### Pushing Forward Polyphenol Extraction in Cool Climate Pinot noir and Cabernet Sauvignon Winemaking

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The quality of red wine depends on the composition of polyphenols influencing wine color and taste. How much must we fear over-extraction, especially of seed tannins, under cool climate conditions? The extraction of polyphenols from grape skins and seeds was investigated for the grape varieties Cabernet Sauvignon and Pinot noir.

### Wednesday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology—Phenolic Extraction Session—CONTINUED

The experimental setup included seed removal, milling the seeds or the cap and returning them back, crushing the whole grapes prior to fermentation, acidification of must, as well as different techniques for cap management. Photometric assays were used to determine total phenols, tannins, and polymeric pigments. Anthocyanins and monomeric phenols were analyzed by HPLC-DAD/FD. Flavan-3-ol dimers and trimers as well as corresponding gallates were quantified by LC-QToF-MS. After bottling, descriptive sensory analysis was performed. The results showed that after seed removal, total phenolics and color intensity decreased. Crushing the seeds significantly increased total phenols, tannins, gallic acid, and, for Pinot noir, also large polymeric pigments. Additionally, a darker wine color was observed, indicating the importance of seed polyphenols for color stability. Acidification of must significantly contributed to wine color due to small polymeric pigments, which were most likely formed by enhanced protonation of acetaldehyde, stimulating the formation of ethylidene-linked structures. Furthermore, the catechin-catechin-gallate concentration was significantly increased due to acidification. This dimer may be released by the acid-catalyzed cleavage of interflavan bonds of higher molecular weight procyanidins. The sensory attributes color intensity, astringency, dry tannins, and bitterness were the differentiating factors among the treatments. Crushing the seeds or the cap increased perception of phenol-related, in-mouth modalities. Must acidification leads to a significantly darker wine color, while wines with seed removal lacked color and phenolic structure.

Funding Support: Ministry of Economy, Transport, Agriculture and Viticulture

#### Wine Tannin in Vinifera-Hybrid Blends is Independent of Blend Timing

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Interspecific hybrid grape cultivars have improved resistance to both biotic and abiotic stresses as compared to Vitis vinifera. However, wines produced from interspecific hybrids are known to have less tannin in red wines due to a combination of lower concentrations of skin tannin and higher concentrations of tannin-binding soluble proteins. One potential approach to increasing final wine tannin of low tannin/high protein hybrids is to blend them with high tannin/low protein V. vinifera. However, the outcome of blending these sources is not obvious, because tannin and protein extraction occur throughout fermentation, and because tannin-protein binding is cooperative. We investigated the effect of cofermentation (CF) and postfermentation (PF) blending of different proportions of Marquette (Mq) and Cabernet Sauvignon (CS) on final wine tannin and protein concentrations. Over two growing seasons, we observed that the blend timing (CF or PF) did not affect final tannin or protein concentrations. In both CF and PF approaches, the final wine tannin concentration could be predicted based on a linear, proportional combination of the tannin concentration of the two monovarietal component wines. Conversely, protein concentrations in the CF and PF blends were generally less than the predicted concentration from the proportional sum of the two components. The loss of wine protein during blending could be well modeled by a Freundlich adsorption isotherm. This work demonstrates both pre- and postfermentation blending with V. vinifera is a viable way to increase tannin content of hybrid wines, at least in situations where the blends have sufficient ratios of tannin to protein.

Funding Support: Iowa Department of Agriculture and Land Stewardship



# Viticulture—Impact of Red Blotch on Grape and Wine Composition Session

# Effects of Grapevine Red Blotch Virus on Grape Development and Resulting Wine Quality

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Grapevine red blotch disease, caused by Grapevine red blotch virus (GRBV) infection, has been found to be widespread in the United States. In 2017, we completed a multiyear study evaluating the disease's effects on Merlot (ME) and Cabernet Sauvignon (CS) on rootstocks 110R or 420A at two locations. Basic chemical data and total anthocyanin content were collected from veraison to harvest and agreed with previous findings that there were considerable differences in sugar and color accumulation at harvest. Then, two mitigation strategies were attempted to minimize the impact of red blotch (RB) disease on final wine quality. First, sequential harvesting of the diseased grapes (RB (+)) was implemented, resulting in a first harvest with the healthy grapes (RB (-), 25 Brix), and a second harvest when the diseased grapes reached 25 Brix (RB (+) 2H). Second, chaptalization of the diseased grapes at first harvest (RB (+) S) was employed to mimic the sugar content of the healthy grapes at harvest. Phenolic profiles of RB (-), RB (+), and RB (+) 2H grapes were determined using reversed-phase high-performance liquid chromatography (RP-HPLC) and a protein precipitation assay, while volatile profiles were determined by solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS). The four wine treatments (RB (-), RB (+), RB (+) S, and RB (+) 2H) were analyzed similarly, in addition to sensory evaluation by descriptive analysis. In general, RB (+) grapes had less total phenolics, anthocyanins, and tannins than RB (-) grapes, and the RB(+) 2H berries had increased levels of all three when compared to the RB (+) berries. For the wines, similar results were observed, indicating that sequential harvest could result in wines with a phenolic composition more closely related to those made from healthy fruit.

Funding Support: American Vineyard Foundation

#### Grapevine Red Blotch Virus: Controlling the Vector

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*Grapevine red blotch virus* (GRBV) is a major concern to grapegrowers and wine producers in the United States due to its reducing Brix, delaying fruit maturity, and having adverse impacts on secondary metabolites that contribute to grape quality. The three-cornered alfalfa hopper (3CAH) was recently shown to vector GRBV in *Vitis vinifera* in a greenhouse study. Previously studied as a pest of alfalfa, peanuts, and soybeans in the southern United States, very little was known about the relationship between 3CAH and grapes that could aid in implementing control measures. *V. vinifera* was tested as a reproductive host. Adult 3CAHs did not survive or reproduce on dormant wood, but reproduction occurred on apical and green shoots. Nymphs could not reach adulthood, suggesting that grapevines are not a good feeding host for 3CAH nymphs. Ten weed and ten cover crop plant species commonly found in vineyards were tested as feeding and reproductive hosts of 3CAH. Adult 3CAHs could reproduce on all plant species that were tested in the legume family, which is in agreement with previous research that found legumes to be preferred hosts of 3CAH. A two-year seasonal

YEARS

### Wednesday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Viticulture—Impact of Red Blotch on Grape and Wine Composition Session—CONTINUED

dynamics study involving weekly groundcover sampling for 3CAH was conducted, and it was found that 3CAHs arrive in the vineyard in late winter, well before budbreak. This finding suggests that 3CAHs are not initially attracted to grapevines in the vineyard, but to the weeds and cover crops. Just prior to bloom is the optimal time to till under leguminous cover crops and weeds to have the most impact on reducing 3CAH populations. The results from these studies can be used to time and implement costeffective management decisions that provide the most significant level of control to reduce the spread of GRBV.

Funding Support: USDA-ARS NP303 National Program funds, CDFA Specialty Crops Block Grant Program agreement SCB14051, CDFA's Pierce's Disease/GWSS Program grant 17-0460-000-SA, California Grapevine Rootstock Improvement Commission

# Effects of Grapevine Red Blotch Disease on Cabernet franc Vine Physiology, Fruit and Wine Quality, and Bud Hardiness

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The effects of red blotch disease (RBD) on Cabernet franc performance were assessed over two years in an Oliver, BC, vineyard where 10% of vines, interspersed throughout the planting, tested positive for Grapevine red blotch virus (GRBV). Physiological and fruiting characteristics of 18 infected and 36 neighboring noninfected vines were measured. RBD reduced photosynthesis rates by 21% in July through September and delayed or prevented autumn leaf fall. Pruning mass was reduced from 681 to 502 g/ vine by RBD. Buds were less hardy in RBD vines, by 2.7°C in mid-November, 2.3°C in early February, and 1.6°C in mid-March. RBD reduced fruit yield by 42%, from 2.05 to 1.18 kg/vine, due to reductions in clusters/vine (21%) and berries/cluster (47%), while berry mass increased from 1.25 to 1.68 g in association with higher numbers of seeds/berry. At harvest, RBD reduced juice soluble solids from 25.9 to 21.8 Brix, increased the TA from 7.9 to 10.2 g/L, and increased pH from 3.38 to 3.61. Berry anthocyanin and tannin concentrations were reduced by RBD, but seed tannin concentration was unaffected. Sensory characteristics were determined for wines made using RBD fruit at 0, 5, 10, 20, and 100% by mass of fruit processed. Wines made from 100% RBD fruit had less blackfruit flavor and aroma, more vegetative flavor and aroma, less body and aftertaste, more acidity, and were lighter in color than wines made with only non-RBD fruit. Other than for red fruit flavor, which decreased with increasing RBD fruit content in the wine, there were no differences in sensory characteristics among wines made with 20% or less RBD fruit. These results reveal the range of negative impacts RBD has on the performance of Cabernet franc under Okanagan Valley growing conditions.

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



#### Viticulture—Impact of Red Blotch on Grape and Wine Composition Session—CONTINUED

#### Effects of Red Blotch Disease on Pinot noir under Oregon's Cool Climate Conditions

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The identification of Grapevine Red Blotch Virus (GRBV) as the causal agent of Grapevine Red Blotch Disease (GRBD) and its attribution to decreased wine quality have garnered industry concern. However, it is unclear how the virus is affecting winegrape quality and vine productivity. Some studies show reduced soluble solids and anthocyanins of infected vines, and this is concerning for Pinot noir producers in Oregon's Willamette Valley, where fruit may struggle to ripen in some seasons. To determine the physiological effects of the virus, a study was conducted in a commercial vineyard with GRBV-positive and -negative vines with varying degrees of symptom expression. Vines were tested to confirm virus status in 2017 and 2018 and monitored regularly for growth measures, vine nutrient status, leaf chlorophyll, leaf photoassimilation, stomatal conductance, yield, and fruit composition over two growing seasons. There were no differences by virus status for leaf chlorophyll, photoassimilation, stomatal conductance, leaf area at veraison, and macro- and micronutrient status over the two years. There were no differences in harvest yields. total soluble solids, total anthocyanins, phenolics, and tannins in either year. Berry pH was greater and titratable acidity reduced in GRBV-positive vines compared to GRBVnegative vines in 2018 but not in 2017. Results from this vineyard showed little to no difference between GRBV-positive and -negative vines. A second vineyard was studied in 2018, and year one results showed delayed ripening, with GRBV-positive vines having less total soluble solids and anthocyanins than GRBV-negative vines. However, there were more total phenolics in GRBV-positive vines at the second site. Vineyard site variability may explain the differences in symptom expression of GRBD vineyards and may relate to plant stressors such as water or nutrients.

Funding Support: Oregon State University College of Agricultural Sciences, Oregon State University Extension Marion County, Oregon Wine Research Institute, and California Department of Food and Agriculture Pierce's Disease Glassy Winged Sharp Shooter Board

#### Deficit Irrigation Reduces Fruit Quality in *Grapevine Red Blotch Virus*-Infected Pinot noir Grapevines

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Although deficit irrigation is used to improve fruit quality in healthy grapevines, it can potentially amplify negative effects of viral disease and reduce fruit quality in *Grapevine red blotch virus* (GRBV)-infected grapevines. Therefore, a two-year field experiment was conducted to understand the interaction between GRBV infection and water deficits on disease development and vine physiology. "Wet" (W) vines were irrigated at 100% of estimated crop evapotranspiration (ET<sub>c</sub>), while "dry" (D) vines received water at 66 and 50% ET<sub>c</sub> in 2017 and 2018, respectively. Healthy (GRBV-) and infected (GRBV+) vines were confirmed by PCR assays. There were no significant effects of irrigation treatment on disease symptom onset. GRBV+ vines had a greater vine water status ( $Y_{stem}$ ) than GRBV-vines (0.12 MPa), but the effects of disease status only appeared postveraison. Consequently, yields were significantly greater in W/GRBV+ vines (+42%). Ripening

YEARS

### Wednesday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Viticulture—Impact of Red Blotch on Grape and Wine Composition Session—CONTINUED

was delayed in GRBV+ vines (0.03 Brix/day), and skin anthocyanin concentration was significantly reduced at harvest in GRBV+ fruit (-30%). Skin tannin concentration was greater in D/GRBV- vines (+15%) but less in D/GRBV+ vines (-12%). In contrast, irrigation treatment did not affect seed tannin concentration, with only disease status providing a significant reduction (-17%). In general, irrigation treatment had little to no effect on berry seed flavonoids compared to disease status. Moreover, deficit irrigation reduced sugar and skin flavonoid concentrations in GRBV+ fruit relative to controls. Although keeping GRBV+ vines well-watered may mitigate some of the negative effects of GRBV infection—and may even improve vine productivity—these results suggest that deficit irrigation may further reduce overall fruit quality in GRBV+ vines. Thus, water deficits should be avoided in GRBV-infected Pinot noir grapevines.

Funding Support: American Vineyard Foundation, Oregon Department of Agriculture, Rogue Valley Winegrowers Association, Oregon Wine Research Institute, Agricultural Research Foundation

#### **Enology–Microbiology of Wine Session**

# Consequences of Inhomogeneously-Distributed Yeast in White Wine Fermentations

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Conditions during white wine fermentation are assumed to be homogenous. In pre- and post-fermentation phases, however, no or little CO<sub>2</sub> is formed and yeast cells are subject to sedimentation. The influence of the inoculation procedure and temperature control on the distribution of yeast cells during white wine fermentation was investigated by empirical and simulative analyses of some yeast-, wine- and tank-related factors. The objective of the study was to explore the consequences of inhomogeneously distributed yeast cells during initial and final fermentation phases on yeast viability and the chemical composition of wine. The sedimentation behavior of yeast cells was investigated in a series of experiments in which wine temperature was recorded at different tank depths. The initial average sedimentation velocity of previously hydrated yeast cells in clarified grape must of 18.5 Brix at 15°C was between 0.3 and 0.6 m/day. With progressing time and distance passed, the sinking rate of yeast cells increased to a maximum observed sedimentation speed of 1.1 to 1.5 m/day. Sedimentation of yeast cells was slower during prefermentation, and when yeast was inoculated through the bunghole, fermentation onset was delayed by several days in lower tank sections. Sampling at different tank depths revealed that top layers were fermenting while bottom layers were still unfermented. CO, formation provided sufficient mixing in the upwards direction, but no agitation was observed in lower layers. Layered fermentation caused temperature gradients of up to 12°C in 7000 L tanks, increased total fermentation duration, caused pyruvate accumulation, and decreased the formation rate of volatile esters, presumably due to inferior nutrient availability in layers with high yeast populations. Cooling jackets in lower parts of tanks and fermentation control based on temperature measurement in the lower section of a fermentation tank amplified preand post-fermentation gradients.

Funding Support: Allianz Industrie Forschung



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#### Enology-Microbiology of Wine Session-CONTINUED

#### Conserved Stress Responses by Yeast during Pinot noir Fermentation Revealed by Regulatory Gene Network Analysis

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To investigate the impact of site on yeast fermentation performance in the context of controlled winemaking conditions across multiple vintages, Pinot noir grapes (clone 667, rootstock 101-14) from 10 sites in California were processed and fermented in quadruplicate at the UC Davis Teaching and Research Winery. Winemaking followed near-commercial conditions, including inoculation with Saccharomyces cerevisiae strain RC212, nitrogen supplementation as needed, and SO, treatment. Across vintages, repeatable site-specific differences in fermentation rate and metabolite concentrations in the finished wines were observed. In 2017, gene expression profiling was used to characterize yeast activity in these fermentations. Unlike DNA sequencing used in microbiome sampling, RNA sequencing captures the metabolically active fraction of the fermentation. Fermentations were dominated by S. cerevisiae RC212 expression, with <1% of S. cerevisiae-expressed genes originating from non-RC212 strains across all vineyards. Non-Saccharomyces fungi were also transcriptionally active across fermentations on day one. Global transcriptome reprogramming occurred during fermentation, with thousands of genes being differentially expressed throughout the five days of sampling. Because transcriptome reprogramming is initiated by transcription factors, transcription factor co-expression networks were built and analyzed to determine conserved regulatory interactions across vineyards. From this analysis, a consensus network of high-confidence interactions was identified that included highly connected genes that are likely key to regulating yeast performance during fermentation. This included genes previously implicated in fine-tuning cellular stress responses, including low glucose levels. Interestingly, interactions between non-Saccharomyces organisms and S. cerevisiae transcription factors were not observed at the vineyard level or in the consensus network, suggesting that the presence of these organisms does not significantly influence gene regulatory networks in the RC212 strain. Future analyses will be aimed at further characterizing and testing these networks to explain differences in yeast fermentation performance and outcomes for grapes originating from different vineyards.

Funding Support: Jackson Family Wines, Lallemand, Gordon and Betty Moore Foundation

# Importance of Measuring the Biomass Composition of Saccharomyces cerevisiae to Model Wine Fermentations Using a Genome-Scale Model

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Two key metabolic activities relevant to industrial wine fermentations are nutrient utilization efficiency and tolerance to high ethanol concentrations exhibited by commercial wine yeast strains. Therefore, studying the details of yeast metabolism is of great interest to develop ways to control stuck or sluggish fermentations. Using a computational method called dynamic flux balance analysis (dFBA) combined with a genome-scale metabolic model of yeast, we simulated growth kinetics of *Saccharomyces cerevisiae* under enological conditions. Using a metabolic model also allowed us to predict flux (reaction rate) values for each metabolic reaction in the cell, which helped visualize metabolic pathways that are important in nutrient

### Wednesday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology-Microbiology of Wine Session-CONTINUED

utilization efficiency of *S. cerevisiae*. However, despite being a robust way to study yeast metabolism, current genome-scale models do not accurately represent the biomass growth equation, resulting in lower predicted maximum cell concentrations than the experimental values, indicating that it is essential to experimentally measure major cellular components. This way, variation in biomass composition between different yeast strains can be assessed, and the resulting improved models used to gain better insight into metabolic differences among strains. In this study, we show that using measured values of DNA, RNA, total lipid, protein, and carbohydrate concentrations in the biomass equation of *Yeast 8.3.3* (developed by the Nielsen research group at Chalmers University) improves our computational predictions. We can also more accurately predict metabolic fluxes for various yeast strains and understand the variation in metabolism between different strains that leads to disparities in nutrient utilization efficiency.

Funding Support: American Vineyard Foundation

# Protection of Wine from Protein Haze Using *Schizosaccharomyces japonicus* Glycoproteins

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The yeast Schizosaccharomyces japonicus releases high concentrations of glycoproteins into the fermentation medium, making it of interest not only for its possible direct use in mixed fermentations with Saccharomyces cerevisiae, but also for the industrial production of glycoproteins that could be purified and subsequently used as additives in winemaking. The possibility of recovering these macromolecules directly from the media allows retention of the native structure of the glucan and, at the same time, avoids the necessity for cell wall enzymatic treatment. There are many advantages associated with the use of such macromolecules during winemaking: improved mouthfeel and fullness, decreased astringency, increased sweetness and roundness, and reduced protein and tartrate instability. We quantified the glycoproteins released by Sch. japonicus after 13 days of alcoholic fermentation of a synthetic, polysaccharide-free grape juice by HPLC. Glycoproteins were then characterized by gel electrophoresis and their carbohydrate composition analyzed by HPLC. The glycoproteins released into the media were then purified by ultrafiltration (10 kDa cut-off), and two different fractions (<50 kDa and >50 kDa) were obtained. After freeze-drying, various concentrations of each fraction were added to a protein-unstable wine. After 13 days of fermentation, the concentration of glycoproteins was ~1.2 g/L. The glycoprotein characterization highlighted the presence of elevated percentages of galactose and mannose and low percentages of glucose and proteins, thus confirming what has been observed in previous studies. Although to different extents, both glycoprotein fractions contributed positively to the protein stability of the wines. Indeed, significant reduction in protein haze induced by heat was observed after adding glycoproteins to the unstable wine. Further studies are needed to evaluate their possible effect on other wine attributes.

Funding Support: Enartis

WEDNESDA ORAL ABSTRACT



#### Viticulture—Managing Pests and Weeds Session

#### Vineyard Problem Management Recommendations: The Vineyard Advisor Mobile App

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The TTU Vineyard Advisor mobile application was developed to deliver timely, knowledge-based recommendations for management of >360 problems afflicting grapes nationwide. The scope of problem coverage includes diseases, insects, mites, nematodes, wildlife, weeds, environmental stresses, and physiological disorders. Recommendations are given for management strategies and action thresholds, natural controls and cultural practices, organic materials, and pesticides. The reference section of each problem cites the peer-reviewed or extension publications from which information and recommendations are derived. The most current list of all pesticides labeled for use on grapes to control a specific problem is retrieved from the EPA label database. Complete pesticide labels are available in PDF format for most products. Two search options are available to the user: search the database for grape problems by common name or search for pesticides labeled for use on grapes. The pesticide search can be done by product name or active ingredient. Weed management recommendations provide cultural practices, general recommendations, and special considerations for difficult-to-control weeds or for weeds that have developed tolerance to herbicides. The most current list of all herbicides labeled for use on grapes to control a specific weed is provided, organized by pre-emergence and post-emergence herbicides. Additional resources provided include ratings of pesticide efficacy in controlling grape diseases, insects, weeds, and other pests; fungicide use requirements (reentry interval, preharvest interval); FRAC resistance class; and additional information on fungicide resistance. External links are provided to the Organic Materials Research Institute database of approved organic products and several pest identification websites. Location-specific links are provided to the user's state agricultural extension service and state pesticide regulatory agency. The TTU Vineyard Advisor mobile application was developed for both iOS and Android operating systems and is available for free download.

Funding Support: American Vineyard Foundation

# Cultivar Susceptibility to Botryosphaeria Dieback and the Relationship to Biochemical Differences in the Wood

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Trunk diseases are caused by fungi that infect grapevine wood primarily through pruning wounds and establish localized infections (cankers) that are often lethal to adjacent fruiting positions. *Neofusicoccum parvum* is one of multiple fungi that causes Botryosphaeria dieback; it is one of the most widespread and virulent trunk pathogens in California. It causes larger cankers in some cultivars than in others. We tested the hypothesis that in the resistant cultivar *Vitis vinifera* Cabernet Sauvignon, primary (sugars, amino acids) and secondary (polyphenolics) metabolite compounds accumulate differently at the canker site than in the relatively susceptible cultivar *V. vinifera* Thompson Seedless. Woody stems of potted plants were wounded and inoculated with *N. parvum*, wounded only, or untouched (control). Metabolites in wood around the inoculation site were measured over a three-month period. Cankers were significantly larger in inoculated Thompson Seedless (57.3 mm) than in inoculated Cabernet Sauvignon (25.7 mm), as expected. Total phenolics and total sugars around

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### Wednesday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Viticulture-Managing Pests and Weeds Session-CONTINUED

the inoculation site were at higher concentrations with infection in Cabernet Sauvignon than in Thompson Seedless and were significantly different after three months. Phenolics have been found to be fungistatic in vitro, and higher concentrations were expected in the resistant cultivar. On the other hand, concentrations of amino acids were lower in infected Cabernet Sauvignon than in Thompson Seedless after two weeks but became similar over time, despite larger cankers in the latter. Lower concentrations of amino acids in Cabernet Sauvignon at two weeks may be associated with limiting *N. parvum* colonization, as the fungus can use the amino acids for nitrogen and carbon.

Funding Support: grant 2012-51181-19954 to K. Baumgartner from the USDA National Institute of Food and Agriculture's Specialty Crops Research Initiative

#### Ounce of Prevention or Pound of Cure: A Comparison of Preventative Practices and Vine Surgery for Trunk-Disease Control

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Grapevine trunk diseases threaten the long-term economic viability of vineyards worldwide. The causal fungi infect pruning wounds with rain, mainly during the dormant season in California. Infections can reduce yield by >90% during the most productive years of a vineyard's lifespan. Preventative practices (pruning-wound protectants, delayed pruning) adopted before symptoms appear (<10 years old) provide significant reduction in losses, while extending the vineyard's productive lifespan. Furthermore, the earlier preventative practices are adopted, the greater the gains. After symptoms emerge (~10 years old), however, preventative practices have limited efficacy. In mature vinevards with trunk diseases, symptomatic vines can benefit from vine surgery. which involves retraining a vine from a shoot above the graft union. In our analysis, we evaluated whether preventative practices and vine surgery are substitutes or complements for improving the profitability and sustainability of winegrape vineyards affected by trunk diseases. We used a simulation model representative of California Cabernet Sauvignon vineyards that incorporated time-varying effects of infection on yields, with and without adoption of preventative practices before symptoms appear and vine surgery after symptoms become apparent. The economic impact of these practices varies with practice, timing of adoption, and vineyard age/disease incidence. We found that vine surgery alone outperforms early-adopted preventative practices, but these practices perform best together, with gains increasing the earlier preventative practices begin. Further, vine surgery achieves the greatest gains when performed on all symptomatic vines beginning in year 11 (no preventative practices) up to year 14 (preventative practices adopted in year 3). Vine surgery with or without preventative practices also substantially extends the profitable lifespan by as much as 100%. Thus, we recommend growers pursue preventative practices in young vineyards and vine surgery after symptoms appear in ~20% of vines or before year 15 to maximize the profitability and sustainability of their vineyards.

Funding Support: United States Department of Agriculture, National Institute of Food and Agriculture, Specialty Crop Research Initiative



#### Viticulture—Managing Pests and Weeds Session—CONTINUED

# Herbicide Reduction Through Organic Undervine Treatment and Its Impact on Malbec Wine Volatile Composition

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Viticultural practices to control the undervine environment have relied on chemical herbicides. The plants they sought to control have become resistant, and many herbicides are now banned or toxic residues have been found in foodstuffs. As a result. we examined alternative methods to reduce/eliminate chemical applications to not only control undervine growth but also to ensure the resulting fruit could produce wines of equal or better quality. Three treatment groups: control (conventional practice) and black or white weedmat were assessed on mature planted Malbec vines in the Hawke's Bay region of New Zealand. Treatments were initiated during dormancy 2015 in a randomized block design, with replicates grouped according to trunk diameter. Viticulture data was collected over the 2016 to 2018 vintages, with small-scale wines (12 kg ferments) produced in 2017 and 2018 through a standardized vinification process to simulate commercial ferments. Aromatic analysis was completed on the wines by gas chromatography-mass spectrometry to elucidate differences that may affect the quality. Additionally, high-performance liquid chromatography (HPLC) was used to quantify select phenolic compounds. There were no statistical differences in vine yield or bunch number over the three seasons. Wines demonstrated aromatic differences by analysis of variance (ANOVA) between the treatment groups and control, with the black weedmat having the greatest number of components increased in 2017. By the 2018 vintage, the aroma components were equivalent to the control. Principal component analysis of the data supports the ANOVA interpretation. Total phenolic content showed no differences; however, HPLC analysis was able to elucidate a few differences. This study demonstrates that sustainable organic methods to control the growth of undervine plants can be done effectively with the application of weedmat. It also highlights that this method of undervine treatment does not hinder yield or perceived wine quality.

Funding Support: Eastern Institute of Technology

### Enology–Wine Chemistry: Oxidations and Aging Session

# Identification of Novel Hydroxy Ketones in Must and Red Wine: Understanding the Formation of Premature Aging Markers

**Ana Peterson**, Alexandre Pons,\* Céline Cholet, Laurence Geny, Philippe Darriet, and Yannick Landais

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Prematurely-aged wines are marked by intense prune and fig aromatic nuances that dominate the desirable bouquet achieved through aging. This aromatic defect is, in part, caused by the presence of 3-methyl-2,4-nonanedione (MND) in concentrations above its detection threshold. Thus, high levels of MND in young red wines can negatively impact quality and aging potential. Determination of MND precursors in wine will improve our ability to understand and predict wine shelf life. The detection of hydroxy ketones bearing the reduced structure of MND in wine suggested possible pathways by which MND is formed and evolves. We report the first identification and quantitation of 2-hydroxy-3-methylnonane-4-one (*syn-* and *anti-*ketol isomers) in must and wine and

\*indicates corresponding author

### Wednesday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology—Wine Chemistry: Oxidations and Aging Session—CONTINUED

combine it with the insight we gained into the evolution of MND and ketols through alcoholic fermentation and wine aging in bottle. Alcoholic fermentation results in a significant decrease in MND and the simultaneous appearance of ketol isomers, indicating that the reduction of MND by yeast yields these ketols. The ketols were synthesized, and their impact on MND formation in wine and their sensory contribution to wine aroma were evaluated. Their detection threshold (195.7 Qg/L) was found to be significantly greater than their concentration range in wine (0.2 to 5.7 Qg/L). A clear impact on the formation of MND in the presence of oxygen was observed. MND and ketols were quantified by HS-SPME-GC-MS (CI, MeOH) in >150 Merlot and Cabernet Sauvignon wines from California, Bordeaux, and Switzerland, from which significant correlations between the two ketones and aging time were obtained. In addition, we found evidence of the presence of ketols in must as glycosylated precursors. This work has greatly improved our knowledge of the formation and evolution of MND, enabling more accurate predictions of the oxidative behavior and aging potential of wines.

Funding Support: Région Aquitaine

# Alternative Reaction Mechanism for Iron(II)/Oxygen Consumption in Wine-like Conditions

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The consumption of oxygen in wine is thought to be related to Fe(II) concentration. This work studied the reaction mechanism of the initial stage of this reaction sequence. The oxidation of tartaric acid by iron(II) under wine-like conditions generates autocatalytic or sigmoidal-like iron(III) formation and dissolved oxygen consumption time traces. These autocatalytic-shaped time traces are distinct with lag, propagation, and termination phases. Each phase has a pH dependence. The propagation phase has the added feature of constant rate or zero-order with respect to Fe(III) and dissolved oxygen. Fe(III) formation and dissolved oxygen consumption that coincides with the depletion of dissolved oxygen. Following dissolved oxygen depletion, hydrogen peroxide time traces result in a maximum accumulation that coincides with the depletion of dissolved oxygen. Following dissolved oxygen depletion, hydrogen peroxide to satisfy the stoichiometric constraints and these experimental time traces. This appears to be the first attempt to apply mathematical rigor and modeling to this fundamental reaction that sets the basis for wine oxidation. The resulting kinetic constants allow for time dependent predictions of oxidation intermediates and products.

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



#### Enology–Wine Chemistry: Oxidations and Aging Session–CONTINUED

#### Elemental Analysis of the Changes in Metal Profiles during Winemaking

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Wine is produced all over the world and its quality depends on various factors, including elemental content. Metals and minerals are needed to grow healthy vines and grapes, while other elements influence the efficiency of alcoholic fermentation. Some elements, such as heavy metals, may not impact the sensory properties of wine but negatively affect human health. It is believed the element content in a wine is a reflection of the environment in which the grapes were grown. However, it is not entirely understood how anthropogenic contributions during winemaking impact the final metal concentration in wine. For this study, the effects of wine processing on element concentrations were analyzed during wine production at the UCD Teaching and Research Winery. Samples were collected from harvest to bottling from two different harvest years. Wines were produced from Cabernet Sauvignon, Merlot, and Chardonnay grape varieties. Sixtytwo elements were measured at each collection timepoint using triple quadrupole inductively coupled plasma-mass spectrometry (ICP-QQQ). Postfermentation processing had the largest impact on element profiles, and we observed both increases and decreases in metal content during winemaking depending on the specific metals and grape varieties evaluated. Concentrations of all elements remained below internationally regulated maxima. By understanding how processing can affect the final elemental content of wine, winemakers will be better equipped to manufacture a more stable, higher quality product.

Funding Support: Research was paid for by University funds and grants.

#### Viticulture—Fruit Composition and Yield Session

#### Autonomous Predictions of Vineyard Yield Through Machine-Learning Modeling of Remote-Sensing and Historical Data

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This work presents a very novel approach to predict vineyard yield at the block level from machine-learning analysis of remote-sensing and historical yield data in a fully autonomous way. The work was performed on a 200-acre ranch in California, divided into 16 blocks. Fifteen years of yield data were available, since the first year of production for the ranch. The ranch was planted with multiple varieties (Cabernet Sauvignon, Merlot, and Cabernet franc), but the rootstock was always 1103P. Even within the same year, variability in yield/block was very large, ranging from ~2 to 10 tons. Weather and soil conditions were also very variable considering the time span of the dataset, the surface of the ranch, and the hillslope conditions. Weather data were obtained from the closest weather station of the California Irrigation and Management System (CIMIS), and soil data obtained from the USDA soil database (SSURGO). Terrain analysis was performed using a digital elevation model to obtain the soil wetness index, aspect, and slope of each vineyard block. Over 600 Landsat 7 images for all years were cloud-filtered, SCRI-off imputed, and atmospherically and topographically corrected. Over 15 different multispectral indexes were computed and integrated over time for all vineyard blocks. A machine-learning meta-ensemble of random forest and extreme gradient boosting was then trained to estimate block yield from this data set of static

### Wednesday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Viticulture—Fruit Composition and Yield Session—CONTINUED

and dynamic data. The machine was subjected to multiple validation routines and was finally able to estimate vineyard yield by the month of July with an error of 0.72 tons/ acre, or less than 15%, in a three-year test set of unobserved data. Interpretation of the model provides new insights about environmental causes of yield variations in space and time. This is a very novel approach that, included in a software-as-a-service for growers, promises to change yield estimation practices in the grape industry.

Funding Support: California State University-startup funds

#### Impact of Sunburn on Ripeness and Concentration of Volatile Aromatic Compounds in Muscat of Alexandria Vineyards

Arturo Calderón-Orellana,\* Ignacio Serra, and Pedro Puentes \*Universidad de Concepción, Vicente Mendez 595, Chile (arcalderon@udec.cl)

Sunburn is a key issue in fresh fruit production, but there is little information regarding its impact in the winegrape industry. Head-trained vines may exhibit high incidence and severity of sunburn due to the close proximity of clusters to the warm soil. This is especially relevant in semiarid regions, which are characterized by high solar radiation and water scarcity. Three non-irrigated head-trained Muscat of Alexandria vineyards, each of a high (~50 cm from the soil) and a low (2 to 10 cm from the soil) fruit zone height, were selected in the Itata Valley, Chile. The objective of this study was to characterize the incidence and severity of sunburn at harvest. There were no differences in Brix, weight, shape, color, and sunburn incidence and severity between fruit zone heights. Approximately 40% of berries exhibited moderate to severe sunburn symptoms. Although sunburn is associated traditionally with greater maturity, the relationship between sunburn and Brix was weak and barely significant. The occurrence of sunburn lesions in berries seems to be related to changes in hue, maximum chlorophyll fluorescence, and the concentration of various aromatic compounds in the skin. The concentration of volatile terpenes tended to be higher when sunburn was more severe. However, this relationship was relatively weak for most volatile aromatic compounds analyzed. These results indicate that fruit zone height may have only a small impact on sunburn incidence and severity. Moreover, the mild response of terpenes to sunburn severity suggests that other antioxidant compounds (e.g., polyphenols) are more relevant to ameliorate the negative influence of chlorophyll degradation in berries affected by sunburn. The high incidence of berry sunburn found in the present study highlights the need for appropriate technical management to reduce the influence of excessive solar radiation and thermal stress on fruit quality.

Funding Support: "Centro de Extensión Vitivinícola del Sur" and the "Cooperativa Agrícola y Vitivinícola Cerro Negro-Quillón Ltda (COOVICEN)"

WEDNESDAY ORAL ABSTRACTS



#### Viticulture—Fruit Composition and Yield Session—CONTINUED

#### Late-Season Climatic and Physiological Factors Interact to Influence Rotundone in Cool-Climate Noiret Winegrapes

Andrew Harner, Michela Centinari,\* Ryan Elias, Bryan Hed, and Justine Vanden Heuvel \*Pennsylvania State University, 218 Tyson Building, University Park, PA 16802 (mzc22@psu.edu)

Rotundone is a grape-derived sesquiterpenoid responsible for the black pepper aroma of several winegrape varieties, including the interspecific red-fruited hybrid Noiret. To evaluate how climate and viticultural factors affect rotundone accumulation in Noiret berries, we compared fruit-set leaf removal to an undefoliated control treatment for two years (2016 to 2017) at seven Noiret vineyards across New York and Pennsylvania with considerable climatic variation. Vineyard mesoclimatic (solar radiation, air temperature, and rainfall) and fruit-zone microclimatic (PAR and berry temperature) parameters were assessed in both years. At each site, vine nutrient and water status, yield parameters, fruit chemistry, and pruning weight were also measured. Rotundone concentrations were quantified at harvest using MDGC-MS and exhibited extensive seasonal and geographical variation. Rotundone was significantly and positively correlated to veraison-to-harvest mesoclimatic variables, namely heat accumulation (quantified as growing degree days, or GDD) and solar radiation. Significant and negative relationships also existed with leaf petiole calcium and magnesium concentrations, measured at veraison. Multiple linear regression was performed, and a four-variable model was constructed ( $r^2 = 0.82$ ) for predictive uses, using postveraison GDD, petiolar calcium concentration, Ravaz index, and petiolar phosphorous concentration as predictor variables. Though this model identifies a suite of important relationships between rotundone and the selected variables, external model validation is necessary before it can be used as a rotundone prediction model. Microclimatic influence was also assessed using a multiple linear regression approach, and a two-variable model was constructed that best explained the observed variation in rotundone concentrations ( $r^2 = 0.54$ ). The two predictor variables selected included preveraison PAR and the percentage of hours with berry temperatures above 30°C from veraison to harvest. These results indicate that cool-climate Noiret rotundone concentrations are strongly related to postveraison mesoclimatic factors, while also being influenced by interactions between these factors and grapevine physiological variables.

Funding Support: Pennsylvania Wine Marketing and Research Board

WEDNESDAY ORAL ABSTRACTS

### Thursday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### **Enology–Wine Macromolecules Session**

#### The Secret Life of Wine Macromolecules

**Keren Bindon**,<sup>\*</sup> Agnieszka Mierczynska-Vasilev, Sijing Li, Bo Teng, Aude Watrelot, Stella Kassara, Marlize Bekker, and Paul Smith \*Australian Wine Research Inst., CNR Hartley Grove and Paratoo Roads, Urrbrae, Adelaide/SA/5064, Australia (keren.bindon@awri.com.au)

Various techniques are used to study interactions between macromolecules. Nanoparticle tracking analysis (NTA) is a particularly useful and accessible way to characterize wine systems. This presentation will showcase how NTA has been used to unlock new knowledge within some important areas of wine chemistry. NTA was first used to investigate the effect of wine matrix components on the aggregation of hazeforming proteins and helped reveal a potential mechanism whereby hydrogen sulfide is released from wines with excess residual copper. The method showed how tannin aggregation changes as grape tannins form polymeric pigments in wine and in response to oxidation. Furthermore, NTA could demonstrate that pectolytic enzyme changes not only the size of wine polysaccharides but also their propensity to aggregate. Complexing of tannins with either commercial mannoprotein or arabinogalactan could also be demonstrated with NTA, showing that aggregation depends on polysaccharide structure. Both the opportunities and limitations of the NTA will be discussed.

Funding Support: Wine Australia

#### Diversity of Total Carbohydrate Composition in Red Wine Polysaccharides in Several Pinot noirs and Other Varieties

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The mouthfeel properties of red wine are crucial characteristics for wine quality. In recent years, wine polysaccharides have seen a growing interest, mainly due to their potential role in mouthfeel and wine stabilization, although their impact in perception and the functional mechanisms remain unclear. Polysaccharides are highly versatile in both molecular weight and composition. Based on our recent study that demonstrated the diversity of the polysaccharide composition of red wines from different varieties and growing regions worldwide, we now have examined two sets of Pinot noirs from Oregon of the same vintage as an example of more closely related samples. The wines were selected according to expert opinions from preliminary studies. They were commercially available and covered lower (<15 USD) and higher (<100 USD) price segments. Compositional analyses were conducted using a molecular weight cut-off (2 kDa) followed by precipitation, acidic methanolysis, silvlation with TMSI, and quantitation of the per-O-trimethylsilvl methyl glycoside derivatives via GC-FID/MS. While the sum of total carbohydrates differed by a factor of ~2 (210 to 470 mg/L eq.), the variation was greater in the monomers. Some monomers, such as arabinose (6 to 30 mol-%), varied more than others, such as rhamnose (10 to 18 mol-%). However, neither polysaccharide content nor total carbohydrate composition revealed obvious patterns distinguishing wines recognized for their mouthfeel, which was reasonably expected considering the complexity of polysaccharide impact on wine quality. Surprisingly though, content and composition of polysaccharides in Oregon Pinot noirs varied to the same extent as in the previous varietal selection, although the latter originated in different varieties, vintages, and continents. So far, monomeric composition can reflect the winemaking process in the contribution of grape- or yeast-derived polysaccharides, but the extent to which this composition affects quality traits such as mouthfeel remains to be investigated.

#### Funding Support: Oregon Wine Research Institute

#### Bold type indicates presenting author



#### Viticulture—Crop Load Management Session

### Impact of Crop Load Management on Terpene Concentration of Gewürztraminer Grapes in the Okanagan Valley

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Crop load management by cluster thinning can improve ripening and the concentrations of key metabolites for grape and wine quality. Timing and degree of cluster thinning alter the efficacy of the treatment. However, little work has been done with white grapes and the impact on terpene content. In this three-year field study conducted in Oliver, B.C., cluster thinning was applied to Gewürztraminer vines at two developmental stages: after fruit set (early thinning) and veraison (late thinning). Three crop levels were targeted: light crop (~7 tonnes/ha), moderate crop (~10.5 tonnes/ha), and high crop (~15 tonnes/ha). Treatments were replicated five times on 10-vine plots according to a randomized block design. Vine leaf area, leaf gas exchange, total soluble solids (TSS), and titratable acidity were measured during berry development and at harvest. Free and glycosylated terpenes were identified and quantified using a HS-SPME-GC-MS and a LI-GC-MS, respectively. Treatments did not affect leaf gas exchange or vine leaf area. TSS concentration during ripening and at harvest was greater in light and moderate crops than in high crop, particularly for early thinning. High crop and light crop-early thinning had the greatest free terpene concentration at harvest; however, a significant interaction between treatment and year was observed. Total glycosylated terpenes at harvest were marginally affected by treatments (p = 0.0631), and light crop-early thinning demonstrated the greatest total glycosylated terpene concentration. Interestingly, total free terpenes were significantly affected by treatments at the sampling before harvest (20 to 21 Brix): light crop-early thinning had a greater concentration than high crop. This result was consistent among the three years. Our study suggests that crop load management can be used as a tool to improve grape terpenes in scenarios where ripening is impaired and grapes cannot reach relatively high sugar levels.

Funding Support: British Columbia Wine and Grape Council, Natural Sciences and Engineering Research, Mitacs, British Columbia Investment Agriculture Foundation

#### Enhanced Fruit Technological Maturity and Altered Flavonoid Metabolic Profile in Merlot by Early Mechanical Defoliation

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Removal of basal leaves around bloom inevitably affects grapevine balance and cluster microclimate conditions, improving fruit quality. Mechanization of this practice allows growers to save time and resources, but to our knowledge, it has not yet been compared with manual application in a cool-climate region, where seasonal temperatures frequently limit fruit technological maturity and phenolic ripening in red *Vitis vinifera* cultivars. In both years of research, berry sugar concentration was greatest with prebloom mechanical treatment (PB-ME). Metabolomics analysis revealed that PB-ME favored accumulation of significantly more di-substituted anthocyanins and flavonols and OH-substituted anthocyanins than manual application. Vine canopy and balance were similar between treatments; therefore, increased ripening with PB-ME was likely due to enhanced microclimate conditions and greater carbon partitioning through a more efficient leaf canopy proximal to the ripening clusters. A follow-up study revealed that fruit from the PB-ME treatment was significantly warmer (+3.2°C) during

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### Thursday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Viticulture—Crop Load Management Session—CONTINUED

veraison and early ripening, likely aiding increased fruit metabolite composition in this treatment. Additionally, data indicated that leaf removal advanced ripening initiation by approximately five days compared to the control, potentially contributing to higher sugar concentrations in fruit at harvest. This information provides new insights in fruit maturation in cool climates and an important strategy for consistently ripening red *V. vinifera* cultivars in cool climates.

Funding Support: Michigan Grape and Wine Industry Council and Project GREEEN

#### Multi-Year Study of the Effects of Cluster Thinning on Vine Performance and Fruit and Wine Composition of Pinot noir

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A three-year study was conducted at a vineyard site in California's Edna Valley to evaluate the physiological and agronomic effects of cluster thinning timing on Pinot noir (clone 115) grapevines. Vines were thinned to one cluster/shoot at three selected time-points during the growing season, then fruit was harvested and made into wine. On average, across all growing seasons, control vines yielded 2.6 kg and thinned vines yielded 1.5 kg, representing a 43% reduction in yield by thinning. No effect of cluster thinning or interaction with growing season was found in vine shoot diameter, internode length, fruit zone light level, or cluster weight. Growing season significantly affected more fruit and wine parameters than did cluster thinning treatment, with interactions between treatment and growing season found in fruit Brix, titratable acidity, and anthocyanins, as well as wine anthocyanins and wine b\* (yellow component). For example, some cluster thinning treatments advanced Brix in 2017 but lowered Brix in 2018, although there were no differences found in ethanol content of the corresponding wines. Cluster thinning treatments increased berry anthocyanins by 43% in 2017 and 103% in 2018 relative to the control, and berry total phenolics 87% in 2017 and 140% in 2018 relative to the control, with no significant differences between cluster thinning treatments applied at different times. However, no corresponding increase in anthocyanins and total phenolics was found in the resulting wines. Only one treatment had more wine anthocyanins than the control in each year, bloom + 4 in 2017 and bloom + 12 in 2018. Only bloom + 4 in 2018 had more total phenolics than the control. Based on current results, reducing Pinot noir yields below 2.6 kg/vine in the Central Coast of California will not increase concentration of desirable compounds in fruit or wine.

Funding Support: California Agricultural Research Institute (ARI)

#### **Enology–Wine Stability Session**

#### Impact of Glutathione-Rich Inactivated Yeast on Wine Stability

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Glutathione-rich inactivated dry yeasts (GSH-IDYs) are claimed to accumulate intracellularly and then release glutathione into the must. Glutathione is beneficial to wine quality, but GSH-IDYs have a greater effect than only to increase the pool of this



### Enology-Wine Stability Session-CONTINUED

antioxidant in the wine. This work estimates quantitatively and qualitatively the ability of yeast derivatives to act as stabilization agents of oxidative processes occurring in wine. An electrophilic derivatization strategy was used to estimate the pool of nucleophilic compounds present in model wines containing a soluble fraction of IDYs with increasing intracellular GSH content. This allowed estimation (in glutathione equivalents) of the fraction that reacted with the electrophilic probe by high-resolution liquid chromatography mass spectrometry (HR-LC-MS). Simultaneously, the DPPH assay specifically developed for wine-like media was used to estimate the total scavenging activity of the IDYs. The DPPH assay provided a first overview of the radical scavenging capacity of IDY soluble fractions. The most efficient IDY was nine times more active than the weakest in scavenging the DPPH radical, with no direct correlation with glutathione concentration. This revealed the likely implication of other compounds involved in the radical stabilization reaction. The derivatization showed that the eight IDYs released different combinations and concentrations of 10 dominant nucleophiles detected in positive ionization mode by HR-LC-MS. A new index was created based on the diversity, abundance, and affinity of each nucleophile for this specific electrophile probe. Comparison to the DPPH scores revealed similar trends, but also more complex relationships between the composition and the activity of such soluble fractions. This new approach, consisting of determining the nucleophilic power of a sample, is also a promising tool in winemaking to estimate the aging potential of a wine before bottling.

Funding Support: Lallemand SAS

# Interactions of Polymeric Compounds in Wine and Potential Benefits of Polysaccharide Addition

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Wine contains a variety of macromolecules categorized as proteins, polysaccharides, or polymeric polyphenols that can influence its physical stability and shelf life. Predicting possible interactions with other additives requires a solid understanding of the physical and chemical principles that control affinities and drive reactions. In most cases, this is based on charge-charge interactions, hydrophobic effects, or a combination of the two. Small-scale trials were conducted in model systems, followed by experiments in a red wine matrix, to identify interaction effects between proteins, tannins, and additives like carboxymethyl cellulose (CMC), with or without added polysaccharides (pectin, glucomannan, mannoprotein, alginate, or combinations of these). Other variables were monitored through pH variation, ultrasound treatments, and omission experiments. The working hypothesis that polysaccharides could be responsible for delaying haze formation after CMC addition to red wines was supported by the present data. Even though polysaccharides interact with proteins and polyphenols, stabilizing them in solution or competing for binding sites, which could result in additional haze formation, their affinity is lower than the ability of CMC to bind to proteins. Whenever CMC is added to the model, it interacts with proteins almost immediately and disrupts all other effects. At pH 3, there is a slow further increase in the presence of polysaccharides in the first days. When polysaccharides and CMC interact, the immediate affinity toward proteins is significantly reduced, indicating a buffering effect of polysaccharide material. This observation is even more significant after ultrasound treatment, indicating the importance of the three-dimensional conformation of one or more macromolecules that slowly unfold over time, causing the delay in reactivity. Experiments in red wine matrix support these initial findings and suggest a positive effect of polysaccharides on physical stability and color retention in red wines.

Funding Support: no external funding

### Thursday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology-Wine Stability Session-CONTINUED

# Monitoring the Polymerization of Red Wine Polyphenols with <sup>13</sup>C-Labeled Acetaldehyde

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Polymerization of polyphenols, especially anthocyanins and flavanols, during red wine maturation is a long-known and well-studied reaction that stabilizes color and attenuates astringency. Among numerous possible reactions, the acetaldehyde-induced coupling of anthocyanins and flavanols is probably the most important reaction during the initial phase of maturation. Although dimers and trimers including an ethylidene bridge have been identified in wine, the fate of these oligomers is still unknown. In this experiment, <sup>13</sup>C-labeled acetaldehyde was added to a young Cabernet Sauvignon directly after malolactic fermentation to induce phenolic polymerization. The stable isotope-labeled acetaldehyde was added in duplicate at two different concentrations (25 and 100 mg/L). The wines were stored for 12 months and the phenolic fraction was extracted by adsorption on XAD7 resin and fractionated by ultra-centrifugation. Polymerization was monitored by spectrophotometric tannin analysis and the stable isotope labeled oligomers were analyzed by UHPLC-DAD-ESI-gToF MS and NMR spectroscopy. While total tannin concentration was not influenced by storage time or added acetaldehyde, the amount of high molecular weight compounds (>10 kDa) yielded after ultra-centrifugation increased slightly after 12 months of storage. The addition of 25 mg/L acetaldehyde led to formation of small polymeric pigments within the first three months, which were subsequently transformed into large polymeric pigments during the rest of storage. Adding 100 mg/L acetaldehyde led to the formation of large polymeric pigments within three months, and the amount of total polymeric pigments even decreased until the end of storage. MS-analyses proofed the successful labeling of the oligomers.

Funding Support: Schormueller foundation, Germany

#### Viticulture—Breeding and Rootstocks Session

#### Growth Chamber Analysis of Dormancy Acclimation Responses within NDSU Grape Germplasm

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In North Dakota, dormancy acclimation responses of grapevines are essential traits necessary to develop genotypes with improved winter hardiness. For consistent production, regional grapevines must adapt to environments with abbreviated growing seasons averaging <150 frost-free days and extreme winter conditions such as the recent, severe 2019 winter polar vortexes. Using a North Carolina Design II factorial mating scheme, the dormancy acclimation responses of breeding lines within the North Dakota State University Grape Germplasm Enhancement Project (NDSU GGEP) were characterized. Weekly measurements were recorded for seedling populations in conjunction with a 0.5 hr decrease in photoperiod from 16 to 10 hr daylight, while temperatures were maintained at 27°C within a regulated growth chamber. Traits monitored included abscission, and ratios derived in synchrony with total shoot



#### Viticulture—Breeding and Rootstocks Session—CONTINUED

growth and total node count. Cumulatively, 16 populations of S<sub>o</sub> seedlings obtained from nine unique parents were phenotyped. Heritability estimates for acclimation responses increased with decreasing photoperiod, and most measured traits reached individual peak estimates between 12.5 and 11.5 hr daylight. Further evaluation of seedling populations identified parents giving rise to progeny with superior acclimation at 12 hr daylight, a photoperiod corresponding to 25 Sept in Cass County, ND, three days prior to the average first frost date. Progeny of both MHND011#1 and ND030#2 performed similarly across multiple monitored traits, when compared to open-pollinated seedlings of Ekre #55, a wild, native *V. riparia* used as a control population with desirable acclimation traits. Consequently, these genotypes are being further characterized for potential use as cold-hardy substitutes within the NDSU GGEP to avoid recurring use of *V. riparia* parents, circumventing some of the species' undesirable fruit size.

Funding Support: USDA Specialty Crop Block Grant Program; Screening of the North Dakota State University Grape Germplasm Collection for Temperature Adaptive Acclimation Response; NOGA #17-375

# Genetic Determination of Vegetative Vigor in a Ramsey Ð Riparia GM Population

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Vigor control achieved by grafting with rootstocks is essential for viticulture, especially in the context of climate change. We studied F1 progeny from a cross between Ramsey and Riparia Gloire de Montpellier, rootstocks that confer high and low vigor, respectively. We hypothesized that vigor, defined as canopy biomass, correlates with growth rate, leaf area, biomass partitioning, and plant hydraulics, and that these variables could be associated with genomic regions. Multiple replicates of 138 F1 seedling plants were evaluated for 60 days in a greenhouse at the University of California, Davis, in 2014 and 2015. Each plant was pruned to a single shoot and watered daily. Shoot growth rate, leaf area, and dry biomass were measured for the complete population, while plant and root hydraulic conductance, stomatal conductance, and water potential were measured for a subset of 50 genotypes. Principle component analysis showed a strong role for shoot growth rate, specific leaf area, plant hydraulics, and partitioning indices for vigor determination. Significant QTLs for leaf area, specific leaf area, and partitioning indices were found on multiple chromosomes. Genes located ~700 kb from each QTL mapping position (350 genes) were interrogated for functional enrichment through the PANTHER online tool, based on the annotation information of the Vitis vinifera PN40024 gene ontology. Using a p-value threshold of 0.05 in a binomial test, key biological processes like photosynthesis, leaf development, light and radiation response, cell plasma membrane, cell wall assembly, sugar metabolism, and nitrogen compounds showed functional enrichment, reinforcing the importance of the QTLs here identified as genetic markers of vigor.

Funding Support: INTA Argentina–Viticulture and Enology, UC Davis

### Thursday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Viticulture—Breeding and Rootstocks Session—CONTINUED

# Field Evaluation of Seven Rootstocks under the Saline Conditions of the San Joaquin Valley of California

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Soil/irrigation salinity is a primary challenge in the western San Joaquin Valley, which accounts for 60% of winegrape production in California. Great uncertainty of available surface water and potentially regulated groundwater pumping jeopardize sustainable grape production. Boron, sodium, and chloride are the main concerns for vine toxicity. Soil/irrigation water boron >1 ppm is regarded to be potentially damaging for grapevine and results in significant yield loss. Petiole/blade tissue analysis is commonly recommended for nutrient assessment. To help improve grapevine production and berry quality under saline conditions, we established a rootstock field trial at a commercial vineyard in 2015 near Cantua Creek in western Fresno County. Seven rootstocks, 1103P, 140 Ruggeri, 1616C, Ramsey, Schwarzmann, GRN-2, and GRN-3, were replicated three times with five vines designated as an experimental unit. Pinot gris budwood was fieldgrafted in 2016 and cane-pruned on a 10-inch cross arm. Soil/irrigation water, petiole/ blade, yield components, and berry composition were determined in 2017 and 2018. 1103P, 140 Ruggeri, and Schwarzmann had the least petiole chloride, and 1103P and GRN-3 had the least petiole/blade boron. GRN-2 and GRN-3 had the most accumulated yield, biggest canopy size and greatest petiole Cl. Vintage had more effect on berry composition than rootstocks. There was a strong positive correlation between juice pH and juice sodium and a strong negative correlation between petiole boron and yield. The two-year results highlighted the challenges of growing grapes under saline conditions and the need for more breeding efforts on salt-resistant rootstocks.

Funding Support: California Grape Rootstock Improvement Commission

# Enology—Vineyard and Winery Impacts on Wine Sensory Session

#### The Importance of Nitrogen Source: Vineyard versus Winery Nitrogen Impacts on Chardonnay and Pinot noir Wine Sensory

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Yeast assimiable nitrogen (YAN) affects alcoholic fermentation. Beyond impacts to alcoholic fermentation, this study also investigates how YAN influences wine sensory attributes. The primary objective was to investigate how the source and concentration of YAN impacted Pinot noir and Chardonnay wine sensory perception. Five treatments were used, including a control (no nitrogen additions), addition of diammonium phosphate (+DAP) or organic nitrogen (+Nutriferm) in the winery, and addition of nitrogen to the soil (+Soil N) or foliage (+Foliar N) in the vineyard. Treatments were established with four replicates for each variety. The +Foliar N treatment did not begin until 2017, while all other treatments were conducted in both 2016 and 2017. Wines were produced using standard protocols and conditions, then underwent sensory evaluation using triangle tests and Napping for aroma and mouthfeel. Results from triangle tests showed Pinot noir and Chardonnay control wines were significantly different from all nitrogen-boosted wines, except for the 2016 Chardonnay with DAP addition. Chardonnay wines produced with different nitrogen supplementation (DAP or



# Enology–Vineyard and Winery Impacts on Wine Sensory Session–continued

Nutriferm) in the winery were also significantly different from each other. In contrast, the 2016 Pinot noir winery nitrogen treatments were not significantly different. For both varieties, +Soil N wines were significantly different from all other treatments in both years, except for the 2017 +foliar N Chardonnay wine. In both the Chardonnay and Pinot noir, Napping showed that treatments were well-grouped and described based on aroma, but not mouthfeel. Based on these results, increasing YAN as well as using different sources of YAN alters the sensory attributes of Chardonnay and Pinot noir wines. Ongoing work includes assessment of juice and must amino acid composition and wine volatile aroma compounds, which will provide further information on how nitrogen composition impacts formation of wine aroma compounds.

Funding Support: Oregon Wine Board, USDA-ARS

# Comparing Pinot noir Wine Sensory Perception and Yield: A Study over Five Vintages (2012 to 2016)

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Historically, the wine industry believes that vine yield has a direct impact on final wine quality, with lower yields equating to higher wine quality. While overcropping can be problematic for fruit ripening and vine health, it is unclear if a direct relationship exists between yield and final wine quality in Pinot noir. Increasing yields while maintaining quality allows for greater winery profitability. Oregon vineyards and winery companies partnered with researchers at Oregon State University to investigate the impact of yield and crop load on final wine quality. Each company evaluated two to five cropthinning treatments by cluster thinning on a cluster/shoot or tons/acre basis during the lag phase of berry development each year. Crop thinning was conducted in replicate blocks, with yield measured at harvest and all data analyzed on a linear row basis as kg/m row. Pinot noir grapes from crop level treatments were harvested and made into wine by participating wineries, following a set winemaking protocol. Descriptive analysis of wines was conducted by a professional winemaker panel after two years of bottle aging. Pinot noir sensory attributes were evaluated using 100 mm visual analog scales. Results were determined using multiple dimensional scaling (MDS) and discriminant analysis and calculated within and across all vintages and within companies. While sensory differences were noted each year, there were no relationships between yield and sensory attributes. There were differences between vintages, but no significant vintage by yield interactions occurred, and only one yield by company interaction was found. These results show that there was no direct impact on final wine quality for the yield range in this study, suggesting that other vineyard aspects, such as achieving good vine balance, may be greater drivers of final wine guality.

Funding Support: Oregon Wine Board

### Thursday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

# Enology—Vineyard and Winery Impacts on Wine Sensory Session—CONTINUED

# Two-Year Study of the Chemical and Sensory Effects of Whole Cluster and Dried Stem Additions in Pinot noir Wines

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The effect of whole-cluster fermentation (WC) at rates of 50 (50% WC) or 100% (100% WC), as well as that of dried stem additions (DS) in Pinot noir (clone 777) from the Edna Valley AVA of California Central Coast was analyzed over two consecutive vintages. Wines were produced in triplicate (n = 3) at industrial scale. In 2016, treatments affected most phenolic and chromatic parameters, with the exception of anthocyanins. After maceration, tannin levels were significantly greater in 100% WC and DS wines, while after nine months of bottle aging, wine color, tannins, and polymeric pigments were greater, again, in 100% WC and DS wines. Additions of WC and DS generally increased pH (by 0.1 units in 2016 and 0.18 units in 2017), and addition of 100% WC consistently increased acetic acid in the final wines over the two vintages. In 2017, WC addition decreased anthocyanins but, together with DS, increased tannins and large polymeric pigments, as in 2016; however, there were no differences in wine color after malolactic fermentation. Wines of both vintages were analyzed by sensory descriptive analysis with a trained panel (2016, n = 8; 2017, n = 10) after three months of bottle aging. Over the two vintages, control wines were generally more colored and acidic and had less aromatics. 50% WC wines had more earthy notes and astringency. 100% WC wines were defined by vegetal, clove, and dark fruit sensory notes, while also being astringent. DS resulted in wines with more brown hue, but produced the most aromatically diverse wines, with berry, dried fruit, red fruit, vegetal, and herbal aromas. Based on these results, the use of dried stems may be a viable practice to increase the aromatic diversity of Pinot noir wines while also increasing the content of condensed tannins in the finished wines.

Funding Support: California Agricultural Research Institute

# Influence of Bunch Rot on Fermentation Kinetics and Sensory in Chardonnay and Petite Sirah

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Greater incidence of fungal infections on winegrapes can negatively impact fermentation behavior and the flavor profile of the finished wine. The most common molds that were shown to have an impact on grape quality are *Botrytis*, *Penicillium*, and *Aspergillus*. The objective of this study was to evaluate the effects of different levels of bunch rot on fermentation kinetics and sensory profiling. Clean and infected grapes were crushed separately to prevent contamination. Six separate 12-gallon fermentations were divided into clean controls, and 5, 10, 15, or 20% rot by weight. Samples were taken daily for FT-MIR analysis. Analytical attributes tracked over the course of fermentation were changes in Brix, titratable acidity, ethanol, volatile acidity, tartaric acid, malic acid, gluconic acid, glucose, and fructose. The goal was to develop an FT-MIR based calibration model to determine the level or concentration of rot that affects the final wine in white and red varieties. The wines were also subjected to difference and preference testing by a trained sensory panel to determine differences between control

#### Bold type indicates presenting author



#### Enology–Vineyard and Winery Impacts on Wine Sensory Session–continued

wines and wines made from infected grapes. Sugar levels were greater in the initial juice containing 20% rot than the control; however, all wines were fermented to dry. Infected grapes consistently recorded higher levels of titratable acidity, volatile acidity, fermentable sugars, and ethanol. Preliminary sensory studies based on preference showed minimal differences at 5, 10, and 15% infection, but 20% infected wines had distinct sensory differences from controls. Vineyards suffer quality loss due to fungal contamination. Currently, visual inspection of fungal contamination is the standard practice to quantify rot quality. Analytical testing to quantify rot percentage, based on the FT-MIR rot indicator matrix, helps create a new industry standard for fungal identification pre- and postharvest, and clarifies sensory differences in fungal-infected wines.

Funding Support: California Winegrape Inspection Advisory Board

#### Effect of Antioxidant Additions at Harvest on Aroma Profiles of Pinot gris, Chardonnay, and Sauvignon blanc Wines

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Many studies have investigated the drivers behind quality Sauvignon blanc wines in terms of varietal thiol content. However, much less is known about their role in Pinot gris and Chardonnay, particularly the effect of antioxidant additions at harvest, despite the popularity of these wines and the interest in the range of styles that can be produced. In this report, different levels of the antioxidants sulfur dioxide and ascorbic acid were added to grapes at harvest, and the effects on the chemical composition and sensory profile of New Zealand Sauvignon blanc, Pinot gris, and Chardonnay wines were examined. For the wine analysis, the aroma compounds included the important varietal thiols, with passionfruit and tropical/ green aromas, given the dominating effect that this family of compounds has on white wine styles. The results showed that high levels of thiols can be produced in Pinot gris and Chardonnay wines, equally high as with Sauvignon blanc, if high antioxidant protection is provided at harvest. As in previous studies, the thiol concentrations can differ by more than four-fold between sites, even with the same treatments, while without added antioxidants, the thiols could not be detected in the final wines. At the same time, the Pinot gris and Chardonnay wines retained their own sensory characters, even when high levels of varietal thiols were present. One risk with the addition of ascorbic acid and sulfur dioxide together in Pinot gris juice is a pinking problem. In further trials, extra elemental sulfur, used as a fungicide and fertilizer, was included with the grapes at harvest, resulting in further increases in varietal thiols. The problem that arose with some wines was unwanted reductive aromas, pointing to the need to control the emergence of hydrogen sulfide at different stages in winemaking.

Funding Support: China Scholarship Council; "New Zealand China Doctoral Research Scholarships"; Constellation Brands

### Thursday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

# Enology—Vineyard and Winery Impacts on Wine Sensory Session—CONTINUED

#### Investigating the Fruitiness Aroma Perception of White Wines from Oregon

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Wine aroma is complex, and many different compounds play a role in fruitiness perception. Fruity aromas such as citrus, pome (pear and apple), tropical fruit, and stone fruit are often used to describe the aroma of white wines. These aromas are highly desired quality attributes by consumers. Therefore, it is essential to explore the sensory perception of white wines from different grape varieties. In this study, fruitiness aroma perception of Pinot gris, Chardonnay, and Viognier wines from Oregon was investigated. A polarized projective mapping (PPM) methodology was adapted. The poles for PPM were aroma standards, representing the four fruity aromas of interest (citrus, pome, tropical fruit, and stone fruit). Over three sensory sessions, trained wine consumers evaluated the fruity aromas of the samples. They were required to smell the wines and participate in check-all-that-apply (CATA), followed by PPM. Results were analyzed using multiple factor analysis (MFA). Overall, wines were grouped by varietal, and panelists could use the poles. Relationships between wines and fruity aromas were found. The outcomes of this work help to better understand the sensory perception of fruity aromas in white wines and, when paired with quantitative chemical analysis, will be able to elucidate the causes of fruity aromas in wine.

Funding Support: American Vineyard Foundation

### Viticulture—Irrigation Session

#### Phloem Contribution to Hydraulic Redistribution in Grapevines

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Redistribution of water transported from grapevine roots in wet soil to roots in dry soil is thought to occur via the xylem and root parenchyma. However, it is unknown how the phloem contributes to hydraulic redistribution. Our hypothesis is that this process in grapevines is in part due to water movement from wet roots to the leaves via the xylem and recycling from the leaves to dry roots via the phloem. This study used deuteriumlabeled water (<sup>2</sup>H<sub>2</sub>O) to trace water flow. Own-rooted Vitis vinifera L. cv. Merlot grapevines were grown in three-way split-root pots. One compartment was irrigated with <sup>2</sup>H<sub>2</sub>O, the second compartment was left to dry with the trunk girdled, and the third compartment was left to dry with the trunk intact. Trunk girdling served to distinguish xylem and phloem water movement. Xylem and phloem sap, trunk and root tissue, and soil samples were collected. Water from each sample was extracted via a cryogenic method and analyzed for deuterium enrichment ( $\delta^2$ H). The  $\delta^2$ H values in root samples from the dry/intact compartment were approximately double those from the dry/girdled compartment. Additionally, leaf petioles were split in half, and one part was immersed in  ${}^{2}H_{a}O$  and the other in EDTA buffer to collect phloem sap. The  ${}^{2}H$  accumulated in the EDTA buffer. These results show that under spatially heterogenous drought stress, some water flows from roots in wet soil to the leaves via the xylem and is then recycled from the leaves via the phloem to roots in dry soil. From a practical perspective, a better



#### Viticulture—Irrigation Session—CONTINUED

understanding of phloem function in whole-plant water transport may be useful in the implementation of irrigation strategies such as partial rootzone drying.

Funding Support: Chateau Ste. Michelle Distinguished Professorship; WA State Grape and Wine Research Program; WSU Graduate School

### Water Stress Response of Eighteen Winegrape Varieties Grown in Eastern Washington

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Interest in understanding the water balance of winegrapes under drought has led to the creation of the isohydric/anisohydric terminology among the different varieties. During a four-year study of dry-down and rewatering cycles, we evaluated the hydraulic behavior of 18 winegrape varieties growing side by side in the same vineyard. Our results show that there may be three distinct major patterns of midday leaf water potential  $(\Psi_i)$ response to soil water availability: linear drop across the entire soil moisture range, linear drop below a threshold of soil moisture, and relative insensitivity to soil moisture. Unlike what is commonly assumed, the stomatal sensitivity to soil water availability did not always mirror the midday  $\Psi_1$  behavior; for example some varieties, like Cabernet franc, show a linear drop of midday  $\Psi_1$  while having a tight stomatal control during soil drought (r = 0.76), while other varieties like Riesling have an insensitive response of midday  $\Psi_{\rm e}$ without having sensitive stomata (r = 0.56). Finally, the slope of the linear regression of midday  $\Psi_1$  on predawn  $\Psi_1$ , as a proxy for the internal regulation of water status, varied between 0.68 for Grenache and 1.2 for Semillon. This shows that, while Semillon behaved as an extremely anisohydric variety, most winegrape varieties in our vineyard had a transpiration sensitivity that was always greater than their hydraulic sensitivity. Since intense yellowing of leaves was recorded in varieties like Cabernet franc, Malbec, and Muscat blanc, these results suggest that the stomatal sensitivity in those varieties may induce carbon starvation during drought. These results may eventually be used by growers to devise variety-specific irrigation management strategies.

Funding Support: Washington State Grape and Wine Research Program, WSU Graduate School

#### Daily Minimum Leaf and Stem Water Potential in Vineyards with Vertical Shoot-Positioning

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Diurnal changes in vine water status and the appropriate time of the day at which to measure leaf and stem water potential (LWP and SWP) were examined in Willamette Valley vineyards employing vertical shoot-positioning (VSP). Measurements of LWP and SWP were performed in Chardonnay and Pinot noir on cloudless days between fruit set and harvest over two years using a pressure chamber. Seven diurnal datasets for LWP and SWP were collected from three VSP vineyards representing warm and cool days and days where vines experienced little water stress or moderate water stress. On warm (1300 hr PST, midday), and remained at this level for a longer duration (1300 to 1700 hr PST) when vines were moderately water-stressed compared to vines under little stress. On cool days, LWP reached the minimum value later in the day (1500 hr PST) in stressed

\*indicates corresponding author

### Thursday National Conference Oral Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Viticulture—Irrigation Session—CONTINUED

and non-stressed vines. SWP reached the daily minimum level late in the day (1500 hr PST) under all conditions, and in one case, increased between late morning and midday before declining to the lowest level at 1500 hr PST. If we consider only those days when it was warm and vines were experiencing moderate water stress, which is the key time to measure vine water status, the daily minimum LWP was stable from 1300 to 1700 hr PST. The corresponding daily minimum SWP was only stable from 1500 to 1700 hr PST. Our findings suggest that viticulturists can increase the time interval to measure LWP up to four hours on warm days, beginning at midday for canopies with VSP. However, SWP measured at midday underestimates the level of vine water stress, and should be determined between 1300 to 1500 hr PST in vineyards using VSP.

Funding Support: USDA-ARS

#### Grapevine Growth and Physiology during Heat and Drought Stress and Recovery

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The frequency and magnitude of heat and drought events are predicted to increase over the coming decades. Although the effect of these factors on grapevine physiology and growth has been investigated separately, little is yet known about their interactions and the variability of these effects among grape cultivars and phenological stages. The aim of this study was to evaluate the response of two grape varieties to heat and drought stress and subsequent recovery at different phenological stages. Pot-grown Cabernet Sauvignon and Riesling were moved to environmentally-controlled growth chambers at bloom, preveraison, and veraison in 2017 and 2018. Different treatments were imposed: control (no stress), water stress, heat stress (10°C above control), and combined water and heat stress. Growth, gas exchange, photochemistry, and leaf water potential were measured before the stress episode, during seven days of stress, and through seven days of recovery. At bloom, water stress decreased gas exchange parameters in both varieties. Combined stress decreased gas exchange only in Riesling. During preveraison, heat stress reduced gas exchange and chlorophyll fluorescence in both young and mature leaves in 2018 but not in 2017. Combined stress drastically decreased most parameters in both varieties in both years. During veraison, drought was the dominant factor affecting most parameters in 2017 and 2018. Additionally, heat stress exacerbated the drought stress effect on the physiological parameters. During the recovery periods, no significant differences were found among treatments in any parameter, indicating that both varieties were able to recover fully from the imposed stresses. Water stress and combined stress decreased shoot length, number of main leaves, lateral leaves, and total leaf area in both varieties.

Funding Support: This work was funded by the Washington State Grape and Wine Research Program (27%) and the Specialty Crop Block Grant Program (73%).

THURSDAY ORAL ABSTRACT9



#### **Enology and Viticulture Research Report Posters**

#### Investigating Filtration Efficiency and Potential Quality Impact of Membrane Filters on Wine

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Filtration can be used at different stages throughout the winemaking process, yet it is mostly associated with quality assurance of the final product. Membrane filtration is commonly used as the last filtration before bottling to ensure microbial stability of wine. Three different types of membrane cartridges of 0.45 Qm were investigated: polyethersulfone (PES), hydrophilic polyvinylidene difluoride (PVDF), and polysulfone (PSF). Each filtration was performed at a constant pressure of 14.5 PSI. A California medium-bodied white blend and a California full-bodied red blend were used in this experiment and compared to a control sample that only went through nominal filtration. Filtration efficiency was determined based on flow rate, clogging tendencies, dissolved oxygen uptake, turbidity, and microbial plating. To evaluate potential impact on wine composition, both volatile (solid-phase microextraction gas chromatography mass spectrometry, SPME-GC-MS) and nonvolatile (Adams-Harbertson assay, reversedphase high performance liquid chromatography (RP-HPLC) with ultraviolet-visible (UV-vis) spectroscopy) profiling of the wines was undertaken. Descriptive analysis was performed to determine the potential sensory impact on wine due to filtration and filter type. In the white blend, only one of 26 sensory attributes was significantly different. In the red blend, three of 30 attributes were significantly different. Sensory differences were seen as minimal and will likely not have a significant influence on perceived wine quality. No significant difference was detected in volatile, nonvolatile, or phenolic content of control and filtered wines within the first six months after bottling. However, differences were observed in filter performance, especially in flow rate and clogging tendencies. Due to membrane filtration's low-to-no impact on wine composition and perceived sensory characteristics, it is recommended that wine filtration should be performed as a stability measure. Decisions on filter choice should be made based on membrane composition and consistency of filter performance.

Funding Support: Fujifilm North America Corporation, Industrial Products Division

# Effect of Organic, Biodynamic, and Conventional Production Processes on the Quality of a Typical Wine

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Environmental values are important drivers for winegrowers because they could represent a credential to offset environmental impacts of wine production. A recent survey on consumer perceptions showed future Old and New World wine markets (USA, Chile, Australia, New Zealand, and South Africa) veering toward organic, reduced carbon-footprint, vegan, and other environmentally friendly products. This study evaluated the impact of organic, biodynamic, and conventional production processes on the quality of a typical wine, such as Chianti DOCG. The global quality of a typical wine was defined by three different profiles: the eligibility profile (chemical characteristics such as alcohol concentration, total acidity, pH, and phenolic concentration), the typicality profile defined by the cultivar (aromatic characteristics that originate from the grapes), and the style profile (characteristics that result from winemaking methods). Fourteen commercial Chianti DOCG wines from the 2016 harvest were selected based on their

#### Enology and Viticulture Research Report Posters—CONTINUED

production management, including organic, biodynamic, and conventional. Chemical analyses (phenol indices, polyphenols, and volatile compounds) and sensory analysis (typicality evaluation and quantitative descriptive analysis) were carried out to define the eligibility and typicality profiles of the wines and to evaluate their correlation with different production techniques. Statistical analysis of the chemical and sensory data emphasized that the different wine production techniques typically had no systematic differences in eligibility and typicality profiles, except for greater color intensity and polyphenol contents in the organic wines. A panel of experts assigned higher typicality scores to one organic, two biodynamic, and two conventional wines, confirming the findings of other authors. Therefore, environmentally friendly production processes may not have necessarily an effect on the recognizability or typicality of a wine.

Funding Support: University of Florence Funds

#### **Residue Behavior of Organochlorine Pesticides during Wine Production**

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The presence of organochlorine pesticides (OCPs) in wine can lead to human exposure. This study investigated the behavior of OCP residues in wine during fermentation. Gas chromatography with electron-capture detection (GC-ECD) was used to detect  $\alpha$ -hexachlorocyclohexane (HCH), hexachlorobenzene (HCB),  $\gamma$ -HCH,  $\gamma$ -chlordane, and  $\alpha$ -chlordane in wine. The results showed that fermentation reduced the residual concentration of OCPs in wine, so we analyzed the processing factors (PFs). The reductions in residue levels during fermentation were due to the activity of the starter. Additionally, white and red wine samples were purchased from local markets, and OCP levels were analyzed. This research provides information for evaluating the risk of wine enology and safety.

Funding Support: A startup fund from Northwest A&F University

# Prefermentation Removal of *Labrusca*-type Odorants from Concord Juice for Its Use in Neutral Red Wines

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Concord grapes and other *Vitis labruscana* cultivars are generally not used for premium wine production due to their distinctive aroma, commonly referred to as being "grapey" or "foxy." We evaluated the selectivity and appropriateness of two prefermentation approaches to reduce characteristic Concord-type odors in finished wines: i) heat treatment/vacuum expansion ("flash détente") of grape must, and ii) nanofiltration followed by resin adsorption (NF-R) of grape juice. Control and treated juices were evaluated for differences in fermentation kinetics and chemical composition (pH, TSS, color, and volatiles). Processing conditions varied in efficacy of methyl anthranilate reduction. NF-R processing reduced levels of key odorants to concentrations below threshold, while flash détente reduced levels of key odorants, but with final concentrations still well above recognition threshold. Fermented wines showed visible differences in color depending on treatment, while the extent of "neutral" character in the finished red wine was determined by the extent to which methyl anthranilate had been successfully removed from the starting juice.

Funding Support: New York State Department of Agriculture & Markets, New York Wine & Grape Foundation, Welch's

#### Bold type indicates presenting author



### Enology and Viticulture Research Report Posters—CONTINUED

#### Preliminary Studies of Redox Titrations in Wine-like Media and Wines

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Reduction and oxidation conditions leading to wine abnormalities are poorly understood. Redox potential throughout the lifetime of a wine may provide insight into the formation of these abnormalities. A method was developed for redox titration with potassium iodate in wine-like solutions and wines. The initial model wine was tartaric acid (5 g/L) and ethanol (15% v/v). One at a time, wine components (Fe(II), Fe(III), catechin, and SO<sub>2</sub>) were added to the initial model wine and titrated. Systemically, these same wine components were assembled and titrated, eventually leading to a more complete wine system with all components present. These "complete" model wine titrations are compared to titrations of commercially available wines. This work is an attempt to understand the active redox species found in wine and to provide insight into the redox buffering ability, which will be important in future redox reactions.

Funding Support: Treasury Wine Estates

#### Evaluating Wine Oxidation Using Spectrophotometric Iron Speciation

Thi Nguyen and Andrew Waterhouse\*

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Wine oxidation is characterized by the redox cycling of iron between its two oxidation states, iron(II) and iron(III), though spectrophotometric speciation has been plaqued by unstable measurements due to alterations to the reduction potential of iron by complexing agents. A production-accessible spectrophotometric method of iron speciation was developed in which two complexing agents, ferrozine and ethylenediaminetetraacetic acid, selective for iron(II) and iron(III) respectively, are used in tandem to stabilize the forms of iron for analysis. Furthermore, bisulfite addition mitigates red wine color that would otherwise interfere with spectrophotometric measurements. The method was used to monitor changes to iron species during continuous aerial oxygenation of several red wines and revealed the logarithmic decline of iron(II) until a plateau is reached, indicating the equilibration of iron oxidation and reduction reactions. However, the precise timing of equilibration and the iron(II):iron(III) ratio at equilibrium were found to vary significantly among the wines studied, suggesting that differential phenolic reduction of iron(III) may be the rate-limiting step governing wine oxidation overall. Ongoing model wine experiments employing this method demonstrate the relationship between iron reduction and oxygen consumption and the effects of compositional parameters such as phenolic content and composition, pH, and copper on reaction rates.

Funding Support: American Vineyard Foundation, ARCS Foundation

WED/THUR POSTER ABSTRACTS

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

#### Elemental Analysis of the Effects of Grapevine Red Blotch Disease in Wine

**Courtney Tanabe**, Arran Rumbaugh, Raul Girardello, Jenny Nelson, Anita Oberholster, and Susan Ebeler\* \*University of California, Davis, Department of Viticulture and Enology, 595 Hilgard Lane, Davis, CA 95616 (seebeler@ucdavis.edu)

Grapevine red blotch virus was discovered in 2008 and has been identified in grapevines across the United States. This virus is the causative agent of grapevine red blotch disease (GRBD), which expresses symptoms of reddening of leaf blades and margins and consistent decreases in sugar and anthocyanin accumulation in grape berries. In addition, recent investigations that focus on berry composition suggest that impacts on secondary metabolites depend on genotype and environmental factors. However, there is a lack of information on element profiles of infected grapes and wine. We investigated the effects of GRBD on element profiles in wines from the 2016 and 2017 harvests. Wines were produced at the UCD Teaching and Research Winery from Cabernet Sauvignon (grafted on two different rootstocks), Merlot, and Chardonnay cultivars. Sixty-one elements were analyzed using a dilute-and-shoot method with a triple quadrupole inductively coupled plasma-mass spectrometer (ICP-QQQ). We observed significant differences in element profiles of wines produced from infected and healthy vines, and the differences depended on the grape variety evaluated. In all cases, increased potassium was observed in wines produced from infected vines. Furthermore, because GRBD is known to cause delays in grape maturation, a sequential harvest was performed on the infected fruit as a possible mitigation strategy. In some cases, elemental profiles of wines from the second harvest remained different compared to the infected and healthy controls.

Funding Support: Research was paid for by University funds and grants. Winemaking was funded through AVF.

#### Prefermentation Skin Contact Temperatures on Aroma Compounds in White Wines Made from La Crescent Grape

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Aroma extract/headspace dilution analysis (AEDA), solid phase microextraction (SPME), and simultaneous multidimensional gas chromatography-mass spectrometryolfactometry (MD-GCMS-O) were used to determine which aroma(s) are dominant in wines made from La Crescent berries. The aim of this study was to determine whether prefermentation skin contact temperatures ( $70^{\circ}F - Lot A$ ,  $45^{\circ}F - Lot B$ , no skin contact treatment – Lot C) spanning 24 hr affected aroma compounds in La Crescent wines. Isoamyl alcohol, isoamyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and sulfur dioxide were found to be key compounds contributing to the aroma of La Crescent wines in all three treatment groups. Statistical analysis for significant differences in aroma compounds due to prefermentation skin contact temperature is currently being investigated. We studied the effect of 24 hr prefermentation skin contact, at two temperatures, on the aroma intensity of La Crescent wines. The sensory panelist could not differentiate between the wines produced by three treatments. We also conducted chemical analysis of aroma compounds using GCMS with sniff port. The results of the study were reported.

Funding Support: Northern Grapes Project




#### Preliminary Findings of Flash Détente Treatment on Marquette Grapes: Wine **Chemical and Aroma Analysis**

Somchai Rice,\* Erin Norton, Lee Lutes, and Kevin Uhl \*Midwest Grape and Wine Industry Institute, Iowa State University, 536 Farm House Lane, Ames, IA 50011 (somchai@iastate.edu)

Flash détente is a thermovinification method during which grapes are heated initially to high temperature, then subjected to a strong vacuum. This treatment causes maximum extraction of many grape chemical components, including anthocyanins, aroma compounds, and tannins. There has been little academic work on the effect of flash détente on resulting wines. In this work, Marquette grapes from Blackstar Farms (Traverse City, MI) were crushed, destemmed, and split into two groups: a control treatment and a flash détente treatment. Following typical fermentation conditions for both treatments, samples were analyzed for aroma and chemical composition. The condensate water from the flash détente treatment was analyzed separately for aroma composition. Differences in all chemical and aroma parameters were observed. Most notably, tannin concentrations were approximately six-fold higher in the flash détente wines. This result leads to further questions regarding the practicality of using flash détente in Northern wine regions to produce red wines with appreciable body and mouthfeel.

Funding Support: Iowa Department of Agriculture and Land Stewardship

#### Influence of Bunch Rot on Fermentation Kinetics and Sensory in **Chardonnay and Petite Sirah**

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Greater incidence of fungal infections on winegrapes can negatively impact fermentation behavior and the flavor profile of the finished wine. The most common molds that were shown to have an impact on grape quality are Botrytis, Penicillium, and Aspergillus. The objective of this study was to evaluate the effects of different levels of bunch rot on fermentation kinetics and sensory profiling. Clean and infected grapes were crushed separately to prevent contamination. Six separate 12-gallon fermentations were divided into clean controls, and 5, 10, 15, or 20% rot by weight. Samples were taken daily for FT-MIR analysis. Analytical attributes tracked over the course of fermentation were changes in Brix, titratable acidity, ethanol, volatile acidity, tartaric acid, malic acid, gluconic acid, glucose, and fructose. The goal was to develop an FT-MIR based calibration model to determine the level or concentration of rot that affects the final wine in white and red varieties. The wines were also subjected to difference and preference testing by a trained sensory panel to determine differences between control wines and wines made from infected grapes. Sugar levels were greater in the initial juice containing 20% rot than the control; however, all wines were fermented to dry. Infected grapes consistently recorded higher levels of titratable acidity, volatile acidity, fermentable sugars, and ethanol. Preliminary sensory studies based on preference showed minimal differences at 5, 10, and 15% infection, but 20% infected wines had distinct sensory differences from controls. Vineyards suffer quality loss due to fungal contamination. Currently, visual inspection of fungal contamination is the standard practice to quantify rot quality. Analytical testing to quantify rot percentage, based on the FT-MIR rot indicator matrix, helps create a new industry standard for fungal identification pre- and postharvest, and clarifies sensory differences in fungal-infected wines.

Funding Support: California Winegrape Inspection Advisory Board

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

## Effect of Antioxidant Additions at Harvest on Aroma Profiles of Pinot gris, Chardonnay, and Sauvignon blanc Wines

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Many studies have investigated the drivers behind quality Sauvignon blanc wines in terms of varietal thiol content. However, much less is known about their role in Pinot gris and Chardonnay, particularly the effect of antioxidant additions at harvest, despite the popularity of these wines and the interest in the range of styles that can be produced. In this report, different levels of the antioxidants sulfur dioxide and ascorbic acid were added to grapes at harvest, and the effects on the chemical composition and sensory profile of New Zealand Sauvignon blanc, Pinot gris, and Chardonnay wines were examined. For the wine analysis, the aroma compounds included the important varietal thiols, with passionfruit and tropical/ green aromas, given the dominating effect that this family of compounds has on white wine styles. The results showed that high levels of thiols can be produced in Pinot gris and Chardonnay wines, equally high as with Sauvignon blanc, if high antioxidant protection is provided at harvest. As in previous studies, the thiol concentrations can differ by more than four-fold between sites, even with the same treatments, while without added antioxidants, the thiols could not be detected in the final wines. At the same time, the Pinot gris and Chardonnay wines retained their own sensory characters, even when high levels of varietal thiols were present. One risk with the addition of ascorbic acid and sulfur dioxide together in Pinot gris juice is a pinking problem. In further trials, extra elemental sulfur, used as a fungicide and fertilizer, was included with the grapes at harvest, resulting in further increases in varietal thiols. The problem that arose with some wines was unwanted reductive aromas, pointing to the need to control the emergence of hydrogen sulfide at different stages in winemaking.

Funding Support: China Scholarship Council; "New Zealand China Doctoral Research Scholarships"; Constellation Brands

#### Investigating the Fruitiness Aroma Perception of White Wines from Oregon Angelica lobbi and Elizabeth Tomasino\*

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Wine aroma is complex, and many different compounds play a role in fruitiness perception. Fruity aromas such as citrus, pome (pear and apple), tropical fruit, and stone fruit are often used to describe the aroma of white wines. These aromas are highly desired quality attributes by consumers. Therefore, it is essential to explore the sensory perception of white wines from different grape varieties. In this study, fruitiness aroma perception of Pinot gris, Chardonnay, and Viognier wines from Oregon was investigated. A polarized projective mapping (PPM) methodology was adapted. The poles for PPM were aroma standards, representing the four fruity aromas of interest (citrus, pome, tropical fruit, and stone fruit). Over three sensory sessions, trained wine consumers evaluated the fruity aromas of the samples. They were required to smell the wines and participate in check-all-that-apply (CATA), followed by PPM. Results were analyzed using multiple factor analysis (MFA). Overall, wines and fruity aromas were found. The outcomes of this work help to better understand the sensory perception of



2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

fruity aromas in white wines and, when paired with quantitative chemical analysis, will be able to elucidate the causes of fruity aromas in wine.

Funding Support: American Vineyard Foundation

## Chemical and Sensory Characterization of Sangiovese Wines from Italy and California

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Sangiovese is the most-cultivated red grape variety in Italy and is certified for the production of several DOCG (Denominazione di Origine Controllata e Garantita), DOC (Denominazione di Origine Controllata), and IGT (Indicazione Geografica Tipica) wines in Tuscany and elsewhere in Italy. According to the California Wine Institute, Sangiovese is also one of the most-cultivated Italian red grape varieties in California. Despite the global distribution of this variety, there is a lack of international studies of Sangiovese grapes and wines. For this reason, the present study aimed to compare commercial Sangiovese wines from 2016 and 2017 harvests, produced in Italy (mostly Tuscany) and California. By combining multiple chemical analyses (e.g., volatile profile, color indices, phenol composition, and elemental profiles) and applying multivariate statistical analyses, it was possible to describe the differences and similarities between the two regions. The results showed that phenols, color indices, and volatile aroma compounds differentiated the wines according to the region of origin. The Italian wines were higher in phenolic content and color intensity, while the California wines had higher levels of volatile aroma fermentation compounds. Similar chemical differences were observed across both vintages. A subset of Californian and Italian wines from the 2017 vintage was also evaluated by a trained sensory panel that described their aroma, taste, and mouthfeel profiles. The descriptive analysis showed that the Italian Sangiovese wines were rated higher in honey, cherry, and floral aromas and were sourer and more astringent. California wines were characterized by red berry, citrus, and bell pepper aromas and were sweeter, with a hotter mouthfeel. The results of this study expand our current knowledge of Sangiovese wines and the contribution of regional characteristics to wine composition.

Funding Support: University of Florence - Department of Agricultural, Food, Environmental and Forestry Sciences and Technologies UC Davis - Department of Viticulture and Enology

#### Sensory Profiles for Two Consecutive Years of Twelve United States West Coast Pinot noir Wines from Different Vineyard Locations

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The overall objective of this study is to investigate the reproducibility of sensory differences among Pinot noir wines derived from a single clone grown on different vineyard locations in the United States West Coast over several years. In each vintage, wines are vinified and consecutively stored under controlled conditions. We anticipate finding sensory differences among the wines from the various sites and

## Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

that these dissimilarities will correlate with phenolic components of these wines. This initial sensory experiment was intended to observe if aging affected the wine within one year by verifying sensory characteristics in two consecutive years from a single vintage. The first 2015 vintage was evaluated in spring 2016 and 2017 by descriptive analysis (DA). No study has yet reported on the effect of vineyard location and wine aging over two consecutive years on taste and mouthfeel characteristics. Phenolic compounds are representing important wine constituents, which include anthocyanins, hydroxycinnamates, and monomeric flavan-3-ols, to correlate with taste and mouthfeel attributes using partial least square regression (PLSR). A two-way ANOVA of shared taste and mouthfeel attributes from the two years (sweet, sour, salty, bitter, astringent, viscous, puckering, and hot) showed no significance for the year and wine interaction. We infer, therefore, that our Pinot noir wines aged similarly on these sensory properties. We conclude that, in the future, sensory analyses on these Pinot noir wines produced and stored under identical conditions can either be performed after the bottling date or a year later. The unique, controlled experimental design, together with the sensory analytical tools (DA) and the phenolics composition, help in understanding the impact of grape growing conditions on wine sensory characteristics.

Funding Support: Jackson Family Ray Rossi Chair Funds UC Davis College of Agricultural & Environmental Sciences

#### Aging Profiles of Four Wine Varieties Stored under Four Different Wine Closures for Three Years

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Understanding the effect of different closures on wine aging has been an ongoing research effort globally. Innovative technical closures avoid the development of "cork taint" and offer a range of controlled oxygen transfer rates for post-bottling storage of different wine styles. The goal of this project was to compare the effects of different closures on the aging characteristics of four wines. The wines under consideration were all of high quality and commercially available. These included a Sauvignon blanc, a barrel-aged Chardonnay, a Pinot noir, and a Cabernet Sauvignon. All wines came from wineries in Napa and/or Sonoma, California, and were aged under controlled temperature for three years. The four closures tested were three new DIAM controlled oxygen transfer rate (OTR) closures and a standard 49 mm natural cork. We studied the chemical characteristics of all wine - closure combinations, namely free and total dioxide levels, color, and phenolic profiles. As expected, overall white wines were more susceptible to the effects of oxidation; thus, their aging patterns were more dependent on the level of oxygen allowed by the different closures we tested. Interestingly, we observed a difference in the effect of oxygen exposure on browning and sulfur dioxide decline among the tested white wines. On the contrary, the chemical profiles of red wines were less impacted by the use of distinctive closures during three years of aging. From these results, we could conclude that red wines after this period of aging are more robust toward OTR-controlled closures, which suggests that the choice of closure type is overall more critical for aging white wines.

Funding Support: DIAM



#### Autonomic Nervous System Responses as a New Sensory Tool to Predict Wine Odorant Valence

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The goal of this study is to look deeper into the physiological responses evoked by wine aromas to use them as new sensory parameters to assess wine quality. Specifically, we aim to create a sensory model that can predict the perceived pleasantness of wine odorants using autonomic nervous system responses. Body signals such as heart rate (electrocardiography, ECG), skin conductance (electrodermal activity, EDA), and facial expressions (electromyography, EMG), were measured using wireless electrodes. Experiments were carried out with 13 subjects smelling four different odorants that can be found in wine and a priori classified into positive and negative (+ or -) valence, namely: 3-isobutyl-methoxy-pyrazine (-), (Z)-3-hexenol (-),  $\alpha$ -pinene (+), and geraniol (+). Odorants were dissolved into mineral oil and presented in 15 mL amber flasks. Each participant smelled the odorants in triplicate, resulting in six trials/valence. To predict the valence of the odorants, features from the physiological signals were extracted and given as an input to binary classifiers: K-nearest neighbor, linear discriminant analysis, and Bayesian linear discriminant analysis. Our results show that it is possible to predict the valence of the odorants using physiological measurements. Using the area under the receiving operating characteristic curve as a performance measurement of the models, the combination of all physiological signals (EMG, EDA, ECG) produces a score greater than 0.85 (where 0.5 corresponds to a classification by chance and 1.0 corresponds to perfect classification). In this sense, these results support the hypothesis that physiological signals evoked by specific wine odorants can inform about their a priori valence. By adding unbiased autonomic responses to sensory evaluation, this technique has the potential to be used in wine quality control and product development to assess the effects of winemaking practices on flavor compounds and consumer preference.

Funding Support: California State University- Agricultural Research Institute; Fresno State Industry Advisory Board; Treasury Wine Estates

## An In-depth Analysis of the Validity of Signal Detection Methods in Wine Sensory Analysis

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Signal detection theory (SDT, denoted by the sensitivity index, d') uses variations in means to quantify the difference between a stimulus of interest and a possible random distracting pattern, usually in the form of a "correct rejection" or "false alarm" application. Initially, the sensitivity index was developed by psychologists to deal with case collected data, but it has since been applied to the food and beverage industry: specifically to sensory science applications dealing with difference and threshold testing. For many reasons, the sensitivity index has become an industry standard for analyzing food sensory data because it measures and estimates fatigue in food sensory subjects. However, there is little literature revisiting the assumptions of the sensitivity index in the context of small sample sizes of 40 or less, as are traditionally used in wine sensory analysis. This research aims to revisit the sensitivity index's mathematical expression, assumptions, and biases to tailor it to the food sensory industry. Current

\*indicates corresponding author

## Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

research assumes that sample size will not impact SDT. This research specifically looked at the signal detection method and its applications with difference testing using a 3-AFC method in wine taste tests. The method was validated in this setting by analyzing the spread and frequencies of calculated d' values when compared across populations with similar statistical identifiers (means, variances, and sample size), which should provide significantly similar d' values. Based on the collected results from multiple sessions of 3-AFC tastings, significant differences in calculated d' values were found, providing some insight into the lack of validity of the method specifically for small sample sizes. Currently, data shows that in a small sample (30 to 45 individuals), the d' calculation is not a consistent measurement of data collection fatigue, and other methods should be considered for post-hoc sensory methods.

Funding Support: McDaniel scholarship for sensory research

#### Cherry to Vanilla: Lignin a Resilient Story of Bourbon Barrels

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Kentucky straight bourbon whiskeys are aged for at least two years in newly charred barrels made from American white oak (Quercus alba). Both the grain mash that is fermented and then distilled and in staves of the barrel that contain lignin are the source of many phenolic compounds with desirable aromas and flavors. In this study, we investigated the staves of bourbon barrels to find how lignin content and composition is altered by whiskey maturation and how variation in distillate could interact with oak lignin to alter extractable lignin moieties from the barrel. We found that the C layer (the layer of the stave in the interior of the barrel which has been charred) had a greater proportion of lignin than the outer layers of the stave that increased further in staves from barrels that had been used to age bourbon. Lignin is a complex biopolymer with many connecting motifs with differences in linkages. The lignin linkages present in the C layer were also different from the outer layers of the barrel but were similar across barrels. The same pattern was observed in the aromatic compounds, which were present at higher levels in the C layers of each barrel. The results presented here show that charring may have dramatic effects on the lignin in the staves, which could have a strong influence on the flavor of the spirit aged in the barrel.

Funding Support: NSF EPSCOR

#### Evaluating the Influence of White Rot Fungi on Volatiles Produced by *Quercus alba* Barrel Staves

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Oak barrels are used in the production of a variety of alcoholic beverages. The charring and toasting process during barrel construction breaks down biopolymers in the wood that are released into the aging spirits. During barrel construction, freshly cut wood is made into staves that weather in the open air where microorganisms degrade the wood. Wood is composed of cellulose, hemicellulose, and lignin, and of these, lignin polymers are the most difficult components to break down. Lignin is a complex, organic polyphenolic polymer that generates many positive flavors such as vanilla, smoky, and fruity, but it is resilient to decomposition. White rot fungi species have the ability to



2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

break down lignin in hardwood and may have the potential to influence the flavors generated from oaked products. In this study, we evaluate the influence of *Lentinula edodes* (Shittake) and *Pleurotus ostreatus* (Oyster) to modify the lignin content by inoculation on American oak staves. Fresh cut staves were inoculated, weathered, kiln-dried, milled, and then toasted by a similar process to that found in a cooperage. Model spirit solutions were used to evaluate the effects that such wood would have in a production environment. Model spirit samplers were evaluated by GC-MS for alterations to lignin-based aromatic compounds in the solution.

Funding Support: NSF EPSCOR

## Chemical Effects of Three Barrel-Bending Alternatives during the Aging of Cabernet Sauvignon Wines in French Oak Barrels

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The purpose of this experiment was to examine the effect of three different bending protocols during cooperage of French oak barrels. Cabernet Sauvignon wines were aged 14 mos in four types of French oak barrels: fire bent and fire toasted (FBFT); water bent and fire toasted (WBFT); and fire bent, fire toasted, and filled with water at 82°C for 12 hrs (FBWF). In addition, neutral barrels (French oak, three uses) were used as control wines, with all treatments conducted in triplicate (n = 3). Phenolics and color parameters were followed at two-month intervals, and 29 key volatile compounds were determined by GC-MS midway during aging. Anthocyanins initially decreased more in control wines, but after 14 mos of barrel aging, all treatments showed equivalent values. No differences in the concentration and evolution of polymeric pigments, tannins, and total phenolics were observed. However, wine color was significantly less in control wines after 14 mos of barrel aging. Furfural, methyl-furfural, hydroxymethyl-furfural, and furfuryl alcohol were all generally lower in control wines, followed by the FBWF treatment. Conversely, no treatment effect was observed for vanillin, guaiacol, eugenol, and cis-lactone. While chemical differences among treatments appear small, two preliminary sensory analysis showed specific treatments effects. Consequently, sensory descriptive analysis will be conducted.

Funding Support: Independent Stave Company

#### Two-Year Study of the Effects of Berry Size and Manipulations of Fermentation Solids in Zinfandel Grapes and Wines

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Zinfandel berries from two adjacent vineyards over two consecutive vintages, 2017 and 2018, were segregated into four berry size classes: raisins (9.6 and 7.9% of distribution), 10 mm (18.5 and 17% of the distribution), 12 mm (30.5 and 27.4% of the distribution), and 14 mm (41.4 and 47.7% of the distribution), including unsorted berries, with all treatments made into wine. Berry surface increased linearly with berry size over the two vintages ( $R^2$  = 0.99 and 0.98), but the solid to liquid ratio decreased with increasing berry size. Larger berries had more skin surface, greater fresh and dry skin and seed weights, and more seeds/berry than smaller berries. With the exception of wine made from raisins (<9 mm), which had 222 and 130% more phenolics and 143 and 122% more polymeric pigments than unsorted berries in 2017 and 2018, respectively, extraction

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#### Enology and Viticulture Research Report Posters—CONTINUED

patterns of anthocyanins and tannins during fermentation were unaffected by berry size. However, wines made from 10 mm berries showed consistently higher content of polymeric pigments. A two-way ANOVA on the wines at pressing indicated clear vintage and berry size effects on most phenolic classes extracted initially into wine. The vintage D berry size interaction was significant for each phenolic class, indicating that berries of the same size over different vintages may nevertheless display differential phenolic extraction. We also manipulated berry size through addition of must and fermentation solids to mimic the solid to liquid ratio of selected berry size classes. We concluded that berry size cannot be compensated in Zinfandel: even though larger berries have a comparatively lower solid to liquid ratio than smaller ones and should be amenable to compensation by saignée or by addition of extra solids, these practices had little or no effect on anthocyanin and tannin extraction.

Funding Support: RSCA

#### Cofermentation, Post-Alcoholic, and Post-Malolactic Fermentation Blending of Malbec and Merlot Wines

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During the winemaking process, wines produced from varieties with complementary levels of anthocyanins and tannins can be blended, either after alcoholic fermentation or after malolactic fermentation. In addition, two grape varieties with complementary anthocyanin and tannin levels can also be cofermented, that is, blended immediately after crushing. In this project, two different grape varieties, Merlot (Mr), which has low anthocyanins and high tannins, and Malbec (Mb), which has high anthocyanins and low tannins, were either (a) cofermented (i.e., blended immediately after crushing the grapes, [Mb/Mr COF]); (b) blended after completion of alcoholic fermentation (Mb/Mr post-AF); or (c) blended after completion of malolactic fermentation (Mb/Mr post-MLF). In addition, monovarietal wines of these two varieties were also made for comparative purposes, with all treatments conducted in triplicate (n = 3). From pressing to post-MLF, color dropped 40% in Mb wines, while the anthocyanin to tannin ratio (A/T) increased from 2 to 4.6. However, the relatively low concentration of tannins in these wines (86 mg/L) did not appear to limit the formation of polymeric pigments (particularly small polymeric pigments) relative to the other treatments. In Mr, color dropped 47%, but the A/T ratio increased from 0.7 to 1, with anthocyanins possibly limiting the formation of polymeric pigments. Mb/Mr COF did not improve color stability, experiencing a 43% color drop after MLF and an increase in the A/T ratio from 1.2 to 1.7. The phenolic and chromatic profile of Mb/Mr post-AF and Mb/Mr post-MLF wines differed slightly, with Mb/Mr post-AF producing marginal improvements in color relative to Mb/Mr COF and Mb/Mr post-MLF. In general, Mb/Mr COF, Mb/Mr post-AF, and Mb/Mr post-MLF equalized the tannin and anthocyanin profile of the resulting wines, resulting in anthocyanin to tannin ratios below 2. Descriptive sensory analysis is currently underway.

Funding Support: California Agricultural Research Institute (ARI)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED



#### Enology and Viticulture Research Report Posters—CONTINUED

#### Grape Skin Insoluble Polysaccharides Affect Red Wine Polyphenol Concentration during Fermentation

**Eri Inoue**, Taiki Igari, Riku Hoshino, Fumie Watanabe-Saito, Masashi Hisamoto, and Tohru Okuda\*

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Tannins and anthocyanins are important compounds in red wine and their extraction mechanisms have been investigated. In general, red wine with a deep reddish color is higher quality than red wine with a light color. There are two types of tannin, one originating in grape skin and the other, in seed. Skin tannin leaves a more positive impression on wine taste than seed tannin. We found that anthocyanin and skin tannin contents decreased during fermentation. However, the reason skin tannin content decreased has not been clarified. In this study, small-scale fermentation of two grape cultivars, Cabernet Sauvignon (CS) and Muscat Bailey A (MBA), was conducted, and anthocyanin, total phenol, and tannin contents in must and skin were measured from the start to the end of fermentation. The changes in contents were observed and compared to elucidate the extraction mechanisms during winemaking. In both cultivars, less-hydrophobic anthocyanins were extracted faster into wine than more-hydrophobic anthocyanins. The anthocyanin content in grape skins in must decreased continuously during fermentation and never increased, but hydrophobic anthocyanins remained in skins after fermentation ended. Although we could not clarify which anthocyanins disappeared during fermentation, hydrophobic anthocyanins in must were not extracted under winemaking conditions. In CS must, total phenol and tannin contents increased with fermentation, but in MBA must, they peaked on day five and decreased thereafter. Total phenol and tannin contents in skin of both cultivars decreased from the start of fermentation to day nine or 10, and increased thereafter. Therefore, it is possible that tannin was re-adsorbed by skin. Even so, more studies are needed because phenols re-adsorbed by skin are only a fraction of the phenols that disappeared during fermentation

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## Concentrations of BSA-Precipitable and BSA-Nonprecipitable Tannins in Japanese Wines

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Tannin is a very important component in red wine. Tannin concentrations vary widely in red wines from several countries, including Japan. Tannin has the unique character of binding with proteins, such as bovine serum albumin (BSA), to form a precipitate. This character is believed to be related to the astringency of tannin. Certain amounts of BSA-non-precipitable tannins (NPT) exist in red wine. We measured total phenols (TP), BSA-precipitable tannins (PT), NPT, BSA-precipitable low molecular weight phenols (PSP), and BSA-non-precipitable low molecular weight phenols (NPSP) after separation by BSA precipitation followed by Toyopearl HW-40 column chromatography. All fractions were quantified by the Folin-Ciocalteau method. Monovarietal wines of Cabernet Sauvignon (n = 18), Merlot (n = 17), and Muscat Bailey A (n = 21) were analyzed. The sum concentration of phenols in these four fractions was consistent with total phenols, confirming good recovery of phenols. For Cabernet Sauvignon, the TP,

### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

PT, and NPT were 2205, 627, and 431 mg/L on average. For Merlot, these were 2207, 583, and 443 mg/L, respectively. For Muscat Bailey A, these were 1211, 191, and 117 mg/L, respectively. NPT concentrations were similar in the same variety. Muscat Bailey A had low concentrations of PT and NPT.

Funding Support: JSPS KAKENHI Grant Number JP17K07812

#### Grape Susceptibility to Smoke Taint at Various Developmental Stages

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With the increasing frequency of wildfires globally, flavor and aroma taints resulting from grapevine exposure to smoke are of considerable concern to grapegrowers and winemakers worldwide. After early-season wildfire events in 2017 in Washington state, there was concern about whether smoke exposure prior to the onset of veraison would result in smoke-related aromas and flavors in subsequent wines. To investigate this, two  $200 \times 18$  ft hoop houses were constructed using 80/20 shade cloth covering two rows of 30 vines each, for use as a "smoke" and a "control" house. Moderate-intensity smoke was applied to the smoke house for 48 consecutive hours using either local rangeland plant materials or pine bark to mimic brushland or forest fires, respectively. Smoke intensity was determined by monitoring PM<sub>10</sub> particle concentrations throughout each smoke exposure. Smoke treatments were applied four weeks preveraison, at the start of veraison, three weeks postveraison, and six weeks postveraison, with the first smoke exposure occurring in mid-July and final smoke exposure occurring less than two weeks before harvest in late September. Fruit from all four treatments was harvested over a two-day period in early October. Wines were made using a WSU standard research red winemaking protocol. Initial sensory evaluation of wines found smoke-related aromas and flavors in the smoke-treated wines for all four exposures, including the preveraison smoke trial, while control wines for each smoke exposure did not exhibit smoke-related off-aromas or flavors. Content of smoke-related volatiles and glycosidic precursors is being determined using GC-MS and LC-QToF-MS.

Funding Support: Washington Wine Commission

#### Investigation of Different Winemaking Protocols to Mitigate Smoke Taint Character in Wine

Sandra Dominguez Suarez, Anita Oberholster,\* Charles Brenneman, Hildegarde Heymann, Caroline Doyle, Arran Rumbaugh, and Raul Girardello \*University of California, Davis, 1 Shields Ave, Davis, CA 95616 (aoberholster@ucdavis.edu)

Almost all research regarding smoke taint has been undertaken in Australia, including vine susceptibility, potential mitigation actions during winemaking to limit smoke taint expression, and potential ways to remove smoke taint in the final wines. Thorough review of published smoke taint research indicated large gaps in knowledge and inconsistent results. The objective of this research was to compare all suggested wine protocols recommended in the current literature using smoke-impacted grapes under identical winemaking conditions, except for the parameter under investigation. All investigations were performed at the UC Davis Research and Teaching Winery. Cabernet Sauvignon grapes were received from three different areas with varied amounts of smoke exposure (Oakville, Alexander Valley, and Silverado Trail). Solid phase micro-extraction gas chromatography mass spectrometry (SPME-GC-MS) and descriptive



analysis were performed to correlate wine composition with smoke taint characteristics. The winemaking variables investigated were the impact of different yeasts, oak additions, and fermentation temperatures. Among other attributes, smokiness and ashy aftertaste were found to be significantly different among the wines, showing a clear difference between wines made from smoke-impacted fruit and control wines made from non-impacted fruit. One yeast showed a significant effect by highlighting the fruitiness in the wines and reducing the ashy aftertaste. Different oak additions were not successful in masking the impact of smoke. Similarly, different fermentation temperatures had no significant impact on smoke expression in the resulting wines. Findings indicate that mitigation strategies during red wine fermentation had a limited impact on the extraction of smoke-taint markers and the expression of smoke taint.

Funding Support: American Vineyard Foundation

#### Wildfires in the South Region of Chile: Lessons and Unanswered Questions from the 2017 Mega Wildfire

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In summer 2017, Chile was affected by one of the most destructive wildfires in its recent history. Many factors have been reported to affect the uptake of smoke aroma by grapevine berries, such as phenological stage, cultivar, and smoke composition and exposure, among others. However, there is little information regarding whether the wildfire location or predominant type of vegetation can modify the aromatic profile and sensory attributes of wines. Wine production in southern Chile is predominantly from bush-trained vines established in dryland areas, where the three main planted cultivars, Muscat of Alexandria, Cinsault, and País, are often surrounded by commercial plantations of Pinus radiata, Eucalyptus globulus, and native forest. To improve understanding of the risks of wildfires in this wine region, six Cinsault vineyards were selected based on distance to the wildfire (three next to the wildfire focus and three 20 km from the focus). Analyses of guaiacol, m-cresol, and 2-methoxy-4-methilphenol in the must and wine, before and after malolactic fermentation, showed that guaiacol was only detected in the vineyards closest to the wildfires. Despite guaiacol concentrations of 92 to 169 Qg/L, about three times the detection threshold for this compound, sensory analyses were unable to recognize whether the wines were made from fruit exposed directly to the smoke. Interestingly, guaiacol concentrations were lower in wines than musts. Guaicaol concentrations were greater in clusters exposed to smoke of P. radiata and Nothofagus obliqua than E. globulus. Lignin and secondary metabolites composition might explain differences found between the effects of different woods.

Funding Support: We would like to thank "Centro de Extensión Vitivinícola del Sur" (Chile) for technical and financial support during this study.

#### UHPLC/Q-TOF Characterization of Volatile-Phenol Glycosides in Smoke-Affected Cabernet Sauvignon Grapes during Winemaking

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Wildfire smoke can be detrimental to the flavor of wine made from grapes harvested during or after smoke exposure. "Smoke taint" results from the accumulation of smokerelated volatile phenols in the berries upon exposure to smoke. These volatile phenols



2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

are glycosylated in the berries to a nonvolatile form, but hydrolysis during winemaking and storage can result in release of the undesirable smoke taint compounds. In this study, we used a novel UHPLC q-TOF MS method to characterize and directly track the abundances of volatile phenols during fermentation of smoke-exposed Cabernet Sauvignon grapes. We tentatively identified over 30 volatile phenol glycosides in smoke-exposed grapes, including the first reports of trisaccharide volatile glycosides. During winemaking, the most pronounced changes in glycosidic abundance occurred during the first half of fermentation with *Saccharomyces cerevisiae*. After completion of winemaking, ~75% of glycosides were still present in the wines. Remaining glycosides are free to acid-hydrolyze over time and continue to influence the aroma of the wine.

Funding Support: University of California, Davis

#### Analysis of Monoterpene Alcohols and Glycosides during the Fermentation of Grapes Using UHPLC Q-TOF MS and HS-SPME-GC/MS

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Monoterpene alcohols are a class of aroma molecules in grapes and wine that exist in both free volatile and glycosidically-bound, nonvolatile forms. The nonvolatile glycoside is a sugar-bound, nonaromatic precursor of the aromatic volatile molecules. It is known that the abundances of glycosides decrease during the winemaking and wine aging processes and correspond with an increase in related free volatile molecules; however, an in-depth and direct analysis of individual glycosides and free volatiles is needed to fully understand the behavior of these compounds during winemaking. In this study, both ultrahigh performance liquid chromatography quadrupole-time of flight mass spectrometry (UHPLC Q-TOF MS) and headspace-solid phase microextraction gas chromatography/mass spectrometry (HS-SMPE GC/MS) were used to study the effect of Saccharomyces cerevisiae on the abundances of glycosides and free volatile monoterpene alcohols during primary fermentation of Merlot and Cabernet Sauvignon wines directly. During fermentation, S. cerevisiae induced changes in both free and bound forms of monoterpene alcohols across 27 fermentations. Of the detectible monoterpene glycosides in both grape varieties, one glycoside showed evidence of microbial enzymatic hydrolysis while four other detected glycosides were unaffected. In addition, two different free volatile monoterpene alcohols, geraniol and linalool, increased during fermentation. These findings suggest that multiple factors, such as hydrolysis of glycosides, acidic rearrangements, or yeast metabolism, may influence the aromatic profile of monoterpene alcohols in finished wines.

Funding Support: University of California, Davis

#### Reverse Osmosis as a Method for Mitigating Smoke Taint

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Smoke exposure to grapes prior to vinification can lead to the presence of smokerelated volatile phenols and associated glycosides. These compounds provide negative sensory attributes to the wines, including ashy, medicinal, and smoky aromas and aftertastes. A method to alleviate this taint prior to bottling is needed to mitigate the negative impacts of smoke exposure in finished wines. Smoke-affected wines from the



ongoing smoke taint project in the Collins lab and affected wines from commercial wineries were treated using a small-scale reverse osmosis (RO) filtration system, in which the smoke taint compounds migrated into a permeate stream. The permeate stream was then passed through carbon filter beds to remove the smoke-taint compounds. The composition of the permeate and retentate streams were analyzed using gas chromatography-mass spectrometry (GC-MS) and ultra-high pressure liquid chromatography-quadrupole time of flight-mass spectrometry (UHPLC-QTOF-MS). Principal component analysis of compositional data collected during an RO time-course study found separation between samples from the permeate and the treated wine. In the treated wine, several smoke taint compounds showed a decline in abundance over the treatment period, characterized by a decrease in peak area. Samples taken before and after the carbon filters became more similar as processing time increased, with peak areas decreasing for pre-filtration samples and increasing for post-filtration samples for several smoke-related compounds. This study serves to evaluate the feasibility of using RO to treat smoke-affected wines and as a comparison study for treatment times between commercial and research smoked wines.

Funding Support: Washington Wine Commission, Washington State University start-up funds

## Characterization of Vineyard-Associated *Saccharomyces* spp. and Phenolic Profiles of Pinot noir Grapes in the Okanagan Valley

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Wine is a product of grape juice fermentation by yeast. Terroir is a growing, popular topic among wine scientists, which describes environmental factors that create a distinctive aroma profile for the resulting wine. This promotes interest in regionalspecific native wine yeast strains (Saccharomyces spp.) and the geographically differential phenotypes of winegrapes. The Okanagan Valley (OV) is a major winemaking region in British Columbia (BC), Canada, and this project focuses on identifying the distribution of vineyard-associated wine yeast strains and identifying the phenolic profile of Pinot noir grapes across three subregions of the OV (Kelowna, Naramata-Penticton, and Oliver-Osoyoos) in the 2017 vintage. Using microsatellite analysis, 10 commercial and 22 potentially indigenous Saccharomyces cerevisiae strains were identified from a sample size of 1200 colonies. Over 94% of the strains were unique to a particular subregion. Saccharomyces uvarum was found in several vineyards, and the microsatellite profiles did not overlap between subregions. The identification of unique Saccharomyces strains in OV subregions suggests the existence of regional-specific wine yeasts. The influence of terroir on the phenolic profile of Pinot noir grapes was assessed by analyzing tannin and anthocyanin content in >2000 berries by UV-vis spectrophotometer and HPLC-UV/Vis-MS/MS, respectively. This research may identify distinctive phenolic features of BC grapes that can be used by local winemakers to produce regional-specialty Pinot noir wine. The indigenous wine yeast strains will be further investigated to create inoculates that guarantee predictable, yet regionalspecific, wine fermentation.

Funding Support: British Columbia Wine Grape Council and Mitacs Accelerate

WED/THUR POSTER ABSTRACTS

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

#### Revealing the Metabolomics of Yeast and Filamentous Fungus in Yeast Biocapsules: A Yeast Immobilization System for Wine

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Yeast biocapsules are a yeast immobilization system, used successfully in wine and biofuel production, which uses inert hyphae of a filamentous fungus as a carrier. Prior studies observed physical attachment of yeast cells to hyphae using electron microscopy; however, the relationship between the two organisms has yet to be determined. A screening occurred of the metabolism of the two organisms in this system, *Saccharomyces cerevisiae* and *Penicillium chrysogenum*, during formation of yeast biocapsules in which both organisms remain viable. Endometabolome of biocapsules (yeast and filamentous fungus together) were compared with yeast and then filamentous fungus separately at four successive time points during the start to the completion of biocapsule formation. The screening revealed differences in 68 compounds. Glycerol increased within yeast cells and biocapsules but decreased in filamentous fungus during the final stages of formation. D-trehalose accumulated less at the final time point in biocapsules than in filamentous fungus and yeasts.

Funding Support: Fulbright

## Population Genomics of *Brettanomyces bruxellensis* Isolates from New Zealand

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Brettanomyces yeasts, responsible for the distinct blend of "phenolic," "barnyard," and "medicinal" aromas known as "Brett" character, are an endemic presence in wine regions around the world. A focus of research in many regions has been to determine whether the perennial challenge of avoiding wine spoilage is linked to genetic variation among these yeasts. In other words, is the efficacy of "Brett" control strategies affected by the strains present in a given winery or region? Previous work in Australia and France has shown a link between particular *B. bruxellensis* genotypes and sulfite tolerance. In this study, 70 B. bruxellensis isolates from eight winemaking regions across New Zealand were genome-sequenced and characterized for their capacity to tolerate sulfite. Phylogenetic analyses determined that NZ isolates belonged to four major strain genotypes groups: genotypes also observed among isolates from Australia, France, Germany, Italy, and the United States. Genotype A, isolated from four NZ winemaking regions, corresponds to sulfite-tolerant Australian strain AWRI1499. Sulfite-tolerance testing confirmed the NZ isolates exhibited this same behavior, though a surprising observation was that the relative proportion of isolates belonging to genotype A was substantially lower in NZ (~18%) than Australia (~90%).

Funding Support: New Zealand Winegrowers



## Differences in Lipid Content and Fatty Acid Composition of Pinot noir Wines from Selected Wine Regions

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Wine lipids originate from grape tissues and yeast cell walls. Lipids contribute to the winemaking process by providing material for yeast growth during fermentation. The role of lipids in wine mouthfeel has yet to be discussed, even though medium-chain fatty acids and their esters have been shown to contribute to organoleptic sensory perception. The role of wine lipids, either acting as mouthfeel impact compounds or in interactions with other components such as phenolics, polysaccharide, and tannins that potentially contribute to taste or mouthfeel perception, is poorly understood. This study examined whether lipids can affect wine mouthfeel characteristics. Pinot noir wines produced from Oregon and France were selected for the chemical analyses. Total lipid content was isolated using liquid-liquid extraction method with chloroform/methanol/ water as the solvent system. Lipids extracted from Pinot noir wines, including free fatty acid, triglyceride, glycophospholipid, and sterols were separated and quantified by high pressure liquid chromatography (HPLC). Fatty acid analysis showed that palmitic, stearic, linoleic, and linolenic acids were the major contributors to fatty acid composition. Principal component analysis was used to study the uniqueness of wines from different regions. The differences in total lipid content, fatty acid composition, and concentration of lipid classes in Pinot noir wine may contribute to the evaluation of wine style and guality. The result of this study will be used in sensory research investigating the impacts of changes in total lipid, fatty acid composition, and lipid classes in a winelike model on mouthfeel perception.

Funding Support: E&J Gallo Winery

#### Yeast and Bacterial Inoculation Influence the Microbial Communities and Sensory Profiles of Barrel-Fermented Chardonnay

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Two important biochemical conversions are involved in winemaking. The first is alcoholic fermentation (AF), when wine yeasts, usually strains of Saccharomyces cerevisiae, convert the sugars in grape juice to ethanol and other secondary metabolites. The second is malolactic fermentation (MLF), conducted by lactic acid bacteria (LAB), which involves the conversion of malic acid (green apple) into lactic acid (creamy). MLF is accompanied by citric acid fermentation, which produces acetic acid (vinegar) and diacetyl (butter). MLF is beneficial to most red wines and some white wines, including Chardonnay, where it can improve a wine's microbial stability and enhance its sensory profile. It is common for winemakers to inoculate grape must with a S. cerevisiae strain for AF and a commercial strain of LAB, commonly Oenococcus oeni, for MLF. Traditionally, however, both AF and MLF began spontaneously and were carried out by yeasts and bacteria that originated from the vineyard and winery. This study set out to investigate the effect that microbial inoculation practices have on the microbial communities and sensory profiles of barrel-fermented Chardonnay. Fermentations were either conducted fully spontaneously, fully inoculated (with both S. cerevisiae and O. oeni), or a combination of the two. S. cerevisiae inoculation was successful, with the inoculated strain dominating, while O. oeni inoculation success was variable, resulting

## Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

in greater bacterial community diversity. Spontaneous fermentations contained a greater microbial diversity and a greater proportion of indigenous yeasts and bacteria. Sensory evaluations of the wines revealed a significant increase in fruity aromas in the spontaneous fermentations, with increased vanilla and buttery characteristics. These results highlight the importance of studying the entire microbial community in fermentations to gain a more complete understanding of the ways in which decisions made at the beginning of the winemaking process can affect the quality of the finished wine.

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

#### Optimizing the Use of Cleaning and Sanitizing Agents for Managing Common Spoilage Microorganisms and Fermentation Soils

**Cory Marx**, Charles Brenneman, and Anita Oberholster\* \*University of California, Davis, 595 Hilgard Lane, Davis, CA 95616 (aoberholster@ucdavis.edu)

Cleaning and sanitation are some of the most critical aspects of winery operations and among the largest uses of time and resources. Therefore, choosing the right chemicals, contact times, and monitoring strategies are important means of optimizing these essential processes. This research seeks to address this issue by adapting the minimum inhibitory concentration (MIC) and minimum biocidal concentration (MBC) assay-frequently used for clinical antimicrobials-to determine minimum chemical concentrations required to inactivate seven common winery spoilage organisms. This protocol was adapted for sessile microbial physiologies as a minimum biofilm eradicating concentration (MBEC assay). Dual-channel fluorescence was employed as a live/dead proxy to assess the question of contact time. Fluorescent dyes could reliably label live and dead populations in standardization experiments and revealed that Saccharomyces cerevisiae populations were inactivated in five min or less under several peracetic acid concentrations. These findings were combined to develop test protocols for use in 2000 L-fermentor cleaning trials. ATP swabbing and plate counts were collected from the tanks to compare protocols. The results of these experiments show that the vulnerable areas of tanks (gaskets and areas in the shadow of spray balls or mechanical agitation) had consistent microbial contamination, regardless of the cleaning protocol or contact time. These must be areas of focus in any cleaning and sanitation protocol, and winemakers must be prudent to develop a system that exceeds the typical visual inspection protocol often employed in the winery environment.

Funding Support: American Vineyard Foundation

## Effect of Oxygen on Fermentation Activity and Aroma in Mixed Starters of Hanseniaspora vineae/Saccharomyces cerevisiae

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The use of *Saccharomyces* and non-*Saccharomyces* strains as mixed starters has potential advantages over pure fermentation due to improved wine complexity based on increased aroma quality. In this work, the effects of oxygen on fermentation performance and volatile aroma of French Colombard wine fermented with *Hanseniaspora vineae* and *Saccharomyces cerevisiae* in sequential inoculations were investigated in 1 L flasks. *H. vineae* 71-97 was first inoculated into juice and aerated for



0, 1, or 2 days, then inoculated with *S. cerevisiae* EC1118 for anaerobic culture thereafter. Although dominated by *S. cerevisiae* at the end of fermentation, aeration allowed *H. vineae* to coexist longer with *S. cerevisiae* in mixed cultures, compared to no aeration, due to improved growth of *H. vineae*. In addition, compared to pure *S. cerevisiae* fermentations of 12, 8, and 8 day fermentation times to dryness, with no aeration, 1 and 2 days aeration, respectively, the mixed culture fermentation products and volatile compounds in aerated mixed fermentations were modified significantly and different from those with no aeration and pure *S. cerevisiae* fermentation. The impact of aeration on yeast physiology goes beyond the first aerated step and influences survival rates and for a short time during the cell growth stage in mixed fermentations is a potential means to increase the aromatic diversity and quality of wine, possibly providing an alternative approach to meet the expectations of wine consumers for diversified aromatic quality.

Funding Support: This work was supported by the China Scholarship Council (CSC)

## Volatile Organic Compounds in Wines Produced by Novel Yeasts from the Chihuahuan Desert

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The use of monocultures of Saccharomyces cerevisiae has brought many advantages to the wine industry, such as rapid and reliable fermentations; however, other features such as regional distinction and vintage variability have diminished. Recent studies have proven that indigenous starters can improve the aromatic complexity of wine and reduce ethanol content; its use in mixtures with S. cerevisiae has given good results without compromising production with stuck fermentations. Novel yeasts from the Chihuahuan desert (Aureobasidium namibiae, Candida apicola, Hanseniaspora uvarum, Candida thaimueangensis, and Hanseniaspora opuntiae) were used to ferment synthetic and natural musts. Chemical analyses such as total acidity, volatile acidity, reducing sugars, and headspace solid phase microextraction gas chromatographymass spectrometry were performed on the prepared wines. Volatile organic compound analysis showed that C. thaimueangensis and C. apicola produce high levels of esters, and Hanseniaspora species produce high levels of carbonyl compounds in synthetic and natural must. Results were analyzed by principal component analysis and analysis of variance to find similar characteristics among them. The results of this study contribute to knowledge about yeast communities associated with desert-grown winegrapes.

Funding Support: CIReNa internal research budged/federal government

#### Implementation of a Rapid Antibody-Based Method to Detect Microorganisms Throughout the Winemaking Process

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Off-flavors produced by spoilage microorganisms should be avoided during fermentation. During spontaneous fermentation, difficult storage conditions, or prolonged transport, it is necessary to detect spoilage microorganisms before population sizes achieve the critical level to produce perceptible off-flavors. Additionally,

#### Enology and Viticulture Research Report Posters—CONTINUED

knowing the composition of microorganisms allows winemakers to reduce treatments such as SO<sub>2</sub>. Until now, most methods to detect microorganisms during fermentation have been cost- or time-intensive. This innovative approach is a rapid, antibody-based test system that provides the winemaker with information about beneficial and harmful microorganisms without laboratory equipment or lengthy waiting periods. Antibodies to detect the genera Pichia, Hanseniaspora, and Brettanomyces were generated. An ELISA test system to evaluate the antibodies showed high sensitivity, beginning at 10Đ cells/ mL, and a high specificity for spoilage yeast. For the detection of spoilage bacteria, monoclonal antibody fragments will be produced to decrease cross-reactions. Specific antibodies for the genera Gluconobacter, Pediococcus, Lactobacillus, and Acetobacter have been generated. All antibodies are designed to detect microorganisms worldwide. Industrial production of the rapid test system will deliver a tool for winemakers and distributive traders to get a view inside the ongoing fermentation within 20 min by using a diagnostic dipstick. Additionally, an application software will quantify the cell counts and give handling guidelines for winemakers. Since the assay procedure is easy to use, very fast, and cost-effective, it can be applied widely at all stages of production. With the help of the antibody-based detection method, the quality of wines can be increased while minimizing economic risks.

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## Impacts of Non-*Saccharomyces* Yeasts on Chemical and Sensory Attributes of Merlot Wine with Reduced Amounts of Alcohol

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Non-Saccharomyces yeasts were investigated for their abilities to metabolize sugar from high Brix musts without forming ethanol. Based on evaluation of 16 different non-Saccharomyces yeasts, five species (Metschnikowia chrysoperlae, Mt. pulcherrima, Meyerozyma guillermondii, Pichia kluyveri, and P. membranifaciens) reduced final alcohol contents of wines when inoculated into grape musts prior to Saccharomyces cerevisae. Additional, larger-scale fermentations (300 L) were conducted using two strains of Mt. pulcherrima (P01A016 or NS-MP) or one of My. guilliermondii (P40D002). Here, Merlot grapes (25.4 Brix, pH 3.50, 4.23 g/L TA) were harvested from a commercial vineyard and crushed with 20 mg/L total SO $_2$  added. Must (37.5 kg) was then transferred into triplicate stainless steel fermenters which were inoculated with non-Saccharomyces yeasts (10<sup>6</sup> cfu/mL) followed by S. cerevisiae D254 (10<sup>6</sup> cfu/mL) after three days. After completion of fermentation, 20 mg/L total SO<sub>2</sub> was added to all wines before bottling. Wines inoculated with Mt. pulcherrima P01A016 and NS-MP contained 13.8% and 13.9% v/v ethanol respectively, ~1% less than those inoculated with S. cerevisiae alone (14.9% v/v). The presence of My. guilliermondii had no impact on production of ethanol but did produce greater amounts of 2- and 3-methylbutyl acetate (1.02 mg/L), 2-phenylethyl acetate (0.073 mg/L), and ethyl acetate (148 mg/L) over other wines. All wines with non-Saccharomyces yeasts yielded higher concentrations of 2-methyl-1-propnaol (61.4 to 70.4 mg/L) and 2- and 3-methyl-1-butanol (137 to 165 mg/L) than those with only S. cerevisiae. Descriptive sensory analysis revealed that wines with Mt. pulcherrima P01A016 or My. guilliermondii were more "fruity," "estery," "berry," and had more "dried fruit" character than those fermented with S. cerevisiae alone. This research indicates possible commercial applications of some yeasts in the production of reduced-alcohol wines with positive sensory attributes.

Funding Support: Washington Wine Advisory Comittee Bold type indicates presenting author

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

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#### Enology and Viticulture Research Report Posters—CONTINUED

#### Indigenous *Saccharomyces* Strains Produce Unique Secondary Metabolite Profiles during Controlled Chardonnay Fermentations

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Each winegrowing region around the world hosts unique communities of indigenous yeasts, which may enter fermentations and contribute to the final flavor profile of wines. Particularly in newer winemaking regions, winemakers are increasingly interested in harnessing the winemaking potential of these indigenous yeasts, as they produce unique aroma- and flavor-active secondary metabolites that can improve wine complexity and produce a wine that can be considered truly local. Currently, the only method of introducing these yeasts to fermentations is by allowing grape musts to ferment uninoculated, or spontaneously, thus encouraging indigenous yeasts from the surrounding environment to enter the fermentation on their own. However, at most wineries, commercial yeast strains dominate the winery environment and quickly out-compete any indigenous yeasts in the fermentation. In the Okanagan Valley wine region of British Columbia, indigenous strains of Saccharomyces uvarum have been isolated from spontaneous fermentations at commercial wineries. It is likely that these strains originated from the vineyard, but have since established themselves as winery residents, able to enter and conduct fermentations year after year. We selected four of these previously isolated S. uvarum strains to test their winemaking potential in a controlled setting against commercial strains. We conducted laboratory-scale fermentations (500 mL) of Chardonnay juice, monitoring fermentation kinetics and sampling throughout fermentation for the production of flavor-active compounds by these yeasts. The flavor profile produced by each yeast was measured by gas chromatography-mass spectrometry (GC-MS). Because S. uvarum strains are known to be cryophilic, fermentations were conducted at both 15 and 25°C to determine the effect of temperature on flavor-active compounds and fermentation performance. This research is of particular interest to winemakers looking to create wines with local character, without the risks associated with spontaneous fermentations.

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

## Proteolytic Activity of *Oenococcus oeni* Isolated from Tucumán, Argentina Wines

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Lactic acid bacteria (LAB) are nutritionally fastidious microorganisms; however, under stress conditions they can develop adaptive mechanisms to avoid nutrient limitation. *Oenococcus oeni* is a LAB frequently found in fermented beverages like wine and cider. There is evidence that this bacterium can hydrolyze proteins via extracellular proteases released under nutritional stress conditions. The presence of proteolytic enzymes has great importance for bacterium growth, to prevent protein haze or precipitation and to release biologically active peptides. The presence of proteolytic activity in *O. oeni* RAM11, a strain isolated from red wine from Tucumán wineries (Argentina), was studied for the first time, and the optimal conditions of enzyme activity was determined. *O. oeni* RAM11 was grown in modified Apple Rogosa medium. The cells were obtained in exponential growth phase and incubated in 0.05 M citrate buffer pH 5.0 for two hours

\*indicates corresponding author

## Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

at 25°C. Proteolytic activity was determined in the supernatants and cells. Enzymatic activity was evaluated using as substrate the proteins present in cider and apple juice at different temperatures, pH, and reaction times. Proteolytic activity was determined using a colorimetric method quantifying the peptides and/or amino acids released. No enzymatic activity was found in the bacterium cellular pellet. However, greater proteolytic activity was found in the supernatant. The released enzyme showed an optimal activity at 30°C, pH 5.0, and four hours of incubation. The residual activity was maintained at 40% at 90°C, and total enzymatic inactivation was reached at 121°C. Under optimal conditions, proteolytic activity of *O. oeni* released 12.16 or 4.99 mg N/L peptides from apple juice or cider, respectively. In this work, the presence of extracellular proteolytic activity was evidenced for the first time in strain RAM11 of *O. oeni* isolated from Colalao del Valle wines.

Funding Support: CONICET

#### Histamine Production by Lactic Acid Bacteria from Tucumán Wines. Development of a New Colorimetric Method

**Pedro Aredes Fernández,**\* Silvana Cecilia Ledesma, and María Cristina Rubio \*Universidad Nacional de Tucumán, Ayacuho 471, 4000, Argentina (pedroaredes@hotmail.com)

Biogenic amines (BA) are organic molecules of low molecular weight produced during the vinification process, mainly by lactic acid bacteria (LAB), which affect human health and quality of the final product. Histamine, the primary BA present in wine, can produce hypotension, itching, facial flushing, headache, and diarrhea. Thirty bacterial isolates from the 2013 vintage in Tucumán, Argentina, were screened for the detection of histidine decarboxylase enzyme (HDC) in agar medium according to Maijala procedures in a commercial decarboxilating Moeller broth medium. Four isolates positive for HDC were grown in commercial Moeller broth for 96 hr at 28°C. The supernatants obtained were quantified for histamine using a new method based on a technique for histamine determination in fish. The procedures were modified and adapted for culture medium and wine. The method was validated using a commercial immuno-enzymatic kit for histamine quantification. The gene coding for the enzyme HDC was evaluated in the selected bacteria using PCR. Identification of positive HDC isolates was carried out through amplification and sequencing of the 16S DNA ribosomal gene, and typification was performed using RAPD. The new method showed a linear relationship (correlation coefficient = 0.988) between color intensity at 496 nm and histamine concentration in the range 0 to 100 mg/L. The pink color development increased with histamine concentration. The supernatants obtained in decarboxylating cultures of the selected LAB contained histamine in different concentrations. The higher concentration of the amine was found in the supernatant obtained from Lactobacillus paracasei AT45 (44.35 mg/L). These results show the presence of Lactobacillus paracasei strains with a functional HDC gene in Tucumán wines. Additionally, an inexpensive and rapid method for histamine quantification in culture medium was developed.

Funding Support: Universidad Nacional De Tucumán-facultad De Bioquimica, quimica Y Farmacia. Conicet



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#### Enology and Viticulture Research Report Posters—CONTINUED

## Antiochratoxigenic Activity of Antifungal Metabolites from *Macfadyena* cynanchoides

**Pedro Aredes Fernández**,\* Gisselle Raquel Apud, and Diego Alejandro Sampietro \*Universidad Nacional de Tucumán, Ayacucho 471, 4000, Argentina (pedroaredes@hotmail.com)

Ochratoxin A (OTA) is a mycotoxin with harmful effects on human and animal health. Aspergillus section Nigri, mainly A. carbonarius and A. niger, are the major species producing OTA in grapes and wine. Special attention is being paid to OTA control in agricultural products because of health risks posed by contaminated food. In recent years, plant extracts and their metabolites have become popular to control mycotoxinproducing fungi. In previous assays, we demonstrated that the dichloromethane extract of Macfadyena cynanchoides stems and their antifungal constituents, identified as 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (lapachol) and 1-hydroxy-4-methyl anthraquinone, show significant antifungal activity against A. niger and A. carbonarius. The aim of this work was to determine the antiochratoxigenic activity of the dichlorometane extract and their antifungal metabolites, alone and in combination with sodium metabisulfite in different sublethal concentrations. A volume of 4 mL grape juice was supplemented with each antifungal substance and inoculated with 5 D 10<sup>3</sup> spores/mL of *A. carbonarius* or *A. niger*, then incubated for six days at 15°C. At the end of incubation, the amount of OTA produced in the supernatant juice was determined by competitive ELISA using the Ridascreen-Fast ochratoxin A kit. The dichloromethane extract and its antifungal quinones produced a partial inhibition in the OTA production. Sodium metabisulfite induced OTA accumulation at the assayed concentrations. However, the natural antifungals completely suppressed OTA accumulation in mixtures with sodium metabisulfite at all concentrations assayed. We can conclude that the dichloromethane extract of *M. cynanchoides* and its antifungal molecules have potential use as a complement to sodium metabisulfite against the Aspergillus species responsible for black grape rots and OTA production.

Funding Support: Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán (UNT). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

#### New and Inexpensive Culture Medium for an Optimal *Oenococcus oeni* Biomass Production with Advantages for Starter Implantation

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Malolactic fermentation (MLF) is a beneficial process in winemaking. It is recommended to use selected starter cultures, mainly *Oenococcus*, to perform a controlled MLF. The primary goal for effective MLF is to achieve successful inoculation and growth of the starter culture in wine. The RAM10 strain of *O. oeni* isolated from Tucumán wines showed good tolerance to stress factors from wine. This work describes the design of an optimal production medium (OPM) to produce biomass of strain RAM10. Different culture media (OPM<sub>1</sub> to OPM<sub>18</sub>) were tested, varying the concentration of grape juice, pH, and ethanol content. The media were incubated at 30°C for 168 hr. Growth was monitored using the OD<sub>560nm</sub>. The data were processed statistically using the Kruskal and Wallis analysis, followed by adjustment of the Gompertz model. Four media with greater logarithmic increments in bacterium biomass (C), shorter duration of the latency phase (DLP), and maximum growth rate (B) were selected. Sterile red wine supplemented with L-malic acid (3.5 g/L) was inoculated with RAM10 previously grown in the different selected

\*indicates corresponding author

## Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

media or in MLO broth at 10<sup>6</sup> CFU/ mL. The wines were incubated at 23°C for 21 days. Bacterial growth and residual malic acid were determined. The RAM10 strain had greater bacterial increase and viability in wine when it was pre-cultured in OPM<sub>7</sub>, showing a higher consumption rate of L-malic acid. It can be concluded that OPM<sub>7</sub> provided the greatest biomass, better adaptation to wine conditions, and efficient malic acid consumption. The selected medium has inexpensive components and is easily available to wineries.

Funding Support: Facultad de Bioquímica, Química y Farmacia. UNT. CONICET

## Influence of *Metschnikowia pulcherrima* on Alcoholic Fermentation and Wine Sensory Profile

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Over the past 50 years, the use of selected starter cultures of Saccharomyces cerevisiae to control alcoholic fermentation has broadened, as it is the safest way to ensure the completion of fermentation and to avoid the production of undesirable off-compounds. Non-Saccharomyces yeasts, naturally predominant in grape must, are thus rapidly outcompeted. Although these species were long viewed as spoilage yeasts, their potential for improving the sensory quality of wines is now acknowledged. Indeed, some non-Saccharomyces yeasts possess some specific metabolism pathway and hydrolytic enzymes for the liberation of varietal aromas. A strain of Metschnikowia pulcherrima was studied in sequential inoculation with S. cerevisiae. The impact of the main components of grape, i.e., concentrations of sugars, nitrogen, and lipids, was tested in synthetic must using a Box-Behnken experimental design. The fermentation kinetics were monitored. and the production of main metabolites and aroma compounds (higher alcohols, acetate and ethyl esters, acids, and thiols) was measured at the end of the fermentation. The comparison between sequential inoculation and pure cultures revealed the benefit of using *M. pulcherrima* in fermentation. The impact and interaction of the parameters were assessed, showing that nitrogen was the nutrient most influencing the sensory and organoleptic profile of wines. These results are of great interest for the management of *M. pulcherrima* in sequential inoculation in alcoholic fermentation. They pave the way to further investigations on both the metabolic and transcriptomic origins of the phenotypic specificities observed and on the interactions taking part between M. pulcherrima and S. cerevisiae.

Funding Support: INRA-UMR SPO, Universite Montpellier, Lallemand SAS

Microvinification as a Method for Predicting Smoke Taint in Wine Glenn McGourty,\* Anita Oberholster, Michael Jones, and Ryan Keiffer \*UCCE Mendocino County, 890 North Bush Street, Ukiah, CA 95482 (gtmcgourty@ucanr.edu)

Microfermentation, even with partially ripened fruit, is an inexpensive and fairly accurate method to determine quickly if fruit has a potential smoke taint problem following exposure to wildfire smoke. Testing the fruit for volatile phenolics (guaiacol, 4-methyl guaiacol) and glycosides (cresols, syringol) is both expensive and not completely predictive, as standards for smoke damage aren't universally accepted. In this study, 20 kg of fruit was sampled and microvinified just postveraison (31 Aug) and again when fruit was near complete ripeness (27 Sept) from 13 Cabernet Sauvignon vineyards representing the major growing regions of Lake County, including Big Valley,



High Valley, Upper Lake, Kelsey Bench, Lower Lake, Red Hills, and Guenoc Valley. A 300 g fruit subsample from each vineyard was analyzed for guaiacol and 4-methyl guaiacol. Nineteen L wine lots were then microvinified, stabilized, and bottled for each vineyard for both sampling dates. The wine was analyzed for volatile phenols, and in Australia, for glycoside compounds (4-methyl syringol, o-cresol, p-cresol, syringol, syringol gentiobioside, methyl syringol gentiobioside, phenol rutinoside, cresol rutinoside, guiaocol rutinoside, and methyl guaiacol rutinoside). A 14-member tasting panel of winemakers evaluated the wines for smoke flavors on 27 Nov. Panel members detected off flavors in both sample sets, and tainted wines correlated strongly with elevated concentrations of volatile phenols and glycosides. Not all wines were affected. Off flavors were much stronger in the wines made from riper fruit, as were the concentration of smoke compounds, by as much as six-fold compared to unfermented fruit. Smoke taint was most perceptible when guaiacol concentration > 10 ppb in wine. Some samples had very low levels of all volatile phenolic compounds and no perceptible smoke flavors in sample wines. We concluded that microfermentations are a quick and predictive method to anticipate smoke taint.

Funding Support: Lake County Wine Grape Commission

## Understanding and Controlling Ice-Nucleating Bacteria to Prevent Frost in Vineyards

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Grape vine tissue can super-cool to -5°C without damage in the absence of icenucleating bacteria, which are catalysts for the formation of frost. Ice-nucleating bacteria are ubiquitous in herbaceous and woody vegetation adjacent to vineyards. They readily colonize seedlings germinating on the vineyard floor in the fall and winter. As green grape tissue emerges in the spring, bacteria migrate from cover crops to the vines. We investigated the use of copper sprays and chemical and mechanical mowing of cover crops to suppress ice-nucleating bacteria as a strategy to prevent frost in two 6-ha plots during the 2017 and 2018 growing seasons. No frost occurred in the field during the trial years. One plot was in a Chardonnay vineyard in the Anderson Valley AVA, and the other was a Cabernet Sauvignon vineyard in the Lake County Red Hills AVA. Bacterial populations were very different between sites. Chemical mowing (glyphosate at 1.2 l/ha) and four weekly copper sprays (copper hydroxide at 1.6 kg/ha.) significantly reduced bacterial populations on emerging grape foliage, averaging 1000 colony forming units (cfu) in copper-treated plots compared to 1,000,000 in the control. Vines growing in areas of the vineyard that were chemically mowed had a similar effect in suppressing foliar bacteria on vines. Under temperature-controlled conditions in the laboratory, fresh grape foliage sampled from copper-treated plots could super cool almost -3°C lower than samples from the control plots without freezing. By contrast, in the Red Hills plot, bud break occurred mid-April, and total bacterial populations were much lower (100 fold) in all treatments. There was no significant treatment effect. Our results suggest that copper sprays and suppressing vineyard floor vegetation can be an effective strategy for frost protection where budbreak is early.

Funding Support: California Dept. of Food and Agriculture Specialty Crop Block Grant

WED/THUR POSTER ABSTRACTS

#### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

Analyses of Smoke-Tainted Wine Using Simultaneous Absorbance-Transmittance and Fluorescence Excitation-Emission Mapping

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When wildfires occur near vineyards, grapes can be impacted by the volatiles in smoke. This can cause "smoke taint" in wine, resulting in smoky aromas and flavors and ashy aftertaste, which can lead to substantial economic loss for producers. Predicting the potential effects that fermentation, storage, and consumption will have on the development of smoke taint in wine typically involves detecting and quantifying various compounds associated with smoke taint. This can involve laborious, timeconsuming sample preparation and chromatography, with the separation or liberation of free volatiles from their bound (glycoconjugated) forms prior to analysis. Reported sensory thresholds of many smoke taint volatiles (alone or in summation) are in the low Qg/L range (generally 30 Qg/L or less), requiring highly sensitive chromatography. Typical smoke-derived compounds include phenol, o-, m- and p-cresol, guaiacol, 4-methylguaiacol, syringol, and glycoconjugates thereof, which can be present at substantially increased concentrations in smoke-exposed grapes. Many of these compounds exhibit significant fluorescence quantum yields and distinct spectral features, leading to this study using the patented absorbance-transmittance and fluorescence excitation-emission mapping (A-TEEM) method. The A-TEEM method is well suited to identify and quantify low concentrations of specific analytes among other compounds at much higher concentrations. In this study, partial least squares regression was used to establish effective limits of detection (<3 Qg/L) for key smoketaint compounds, notably including resolution of the cresol isomers. The A-TEEM method may thus be used with sample treatment to quantify free and bound volatiles for smoke-taint evaluation as a chromatography alternative. Further, owing to the significant contribution of smoke-taint compounds to the overall A-TEEM molecular fingerprint within a background of other wine phenolics, smoke-tainted wines may be rapidly (<30 sec) and sensitively detected using multivariate classification techniques including support vector machine and partial least squares discriminant analyses.

Funding Support: HORIBA Instruments, Inc. and Australian Research Council Training Centre for Innovative Wine Production, with support from Wine Australia and industry partners, and the School of Agriculture, Food and Wine, The University of Adelaide.

## Hydrogen Sulfide Generation from Sulfur Dioxide in the Presence of Aluminum in Acidic Solutions such as Wine and Cider

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Hydrogen sulfide ( $H_2S$ ) can be generated when sulfur dioxide ( $SO_2$ ) is reduced in the presence of AI (O) under acidic conditions. Fermented products, including wines, ciders, or other acidic,  $SO_2$ -containing products such as model wines, can form  $H_2S$  when in contact with oxidizing metallic aluminum. Other forms of AI such as oxides or salts do not generate the reductive, electron-liberating potential of corroding AI to generate  $H_2S$  from sulfites.  $H_2S$  production is thermodynamically strongly favored and other VSCs are not generated. In this work, model and white wines containing molecular  $SO_2$  proceeded to produce measurable and sensorially significant amounts of  $H_2S$ . Red wines were also tested, but inconsistently produced  $H_2S$ , likely due to higher



pH and lower molecular  $SO_2$  concentration and/or other complexing factors. This work illustrates the relationship among sulfides and their precursor sulfites, pH, AI, and some potential complexing factors in red wines.

Funding Support: Ball Corporation

## Effects of Prefermentation Additions on Yeast Volatile Aromas and Thiols in Sauvignon blanc and Chardonnay

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Volatile compounds in wines depend on several factors, but mostly on yeast metabolism. Nutritional differences of musts affected yeast production of aroma and flavor compounds. Amino acids and grape-derived precursors are important to the final wine sensory attributes. The aim of this study was to examine the influence of different yeasts and additions of complex nutrients (yeast autolysate) and enzyme on two widely-used grape varieties, Sauvignon blanc and Chardonnay. We measured the different production of thiols, higher alcohols, acetate and ethyl esters. The results demonstrated how different products can be obtained with different combinations of these commercially available products, giving a better idea to winemakers what they can and cannot achieve.

Funding Support: AEB

#### Comparison of Nitrogen Uptake, Fermentation, Dynamics, Efficiency, and Sensory Factors in Wine Depending on Production Method

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Over the past six years, 2B has carried out studies and trials to evaluate differences in fermentation behavior and other features contributing to wine quality and flavor, comparing yeasts that are certified organic-produced versus yeasts from conventional production. Organically produced yeast can contribute to wine quality and flavor without industrial techniques of hybridization or gene engineering, and are therefore a competitive alternative for large- and small-scale production. The presentation shows results of thesis studies and comprehensive field trials in practical scale from various countries. The findings and results of the studies showed the opportunities and benefits of organic principals and methods for any winemaking process. The studies also proved that organic methods and organically produced fermentation products are no compromise to the safety of the winemaking process. The overall organic approach does create more value for the final wines and is not necessarily an ethical approach only. In addition, the certified organic production methods are far more environmentally friendly, economically competitive, and meet the increasing end customers' requests for sustainable production of quality goods.

Funding Support: 2B FermControl GmbH 79206 Breisach Germany

#### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

#### Foliar Urea Application to Increase Berry Yeast Assimilable Nitrogen in Cabernet franc and Sauvignon blanc

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Growing grapes for adequate yeast assimilable nitrogen (YAN) for fermentation can be a challenge. High rates of N fertilizer applied during the growing season can increase berry YAN but may result in undesirable vegetative flavors and aromas in the wine and can also cause excess vine vigor. Cabernet franc and Sauvignon blanc grown on sandy loam soils in the Okanagan Valley of British Columbia were treated with split foliar applications of 2% urea beginning just prior to veraison. All vines received 40 kg/ ha N broadcast at budbreak and 10 kg/ha at flowering. The four treatments included no additional N, an additional 20 kg/ha N broadcast at fruit set, and two or three foliar applications of 2% urea. The foliar urea applications were applied every two to three weeks beginning in early August. Yield components and berry juice soluble solids, titratable acidity, and pH were not affected by the treatments, except in Sauvignon blanc treated with three urea applications, which had a slightly lower juice pH. Berry YAN in Cabernet franc increased by up to 30% in response to three foliar urea applications. In Sauvignon blanc, there were no treatment effects on YAN. This may be due to a compressed ripening period combined with the late first urea application. This suggests that timing urea applications is important in Sauvignon blanc for N uptake into berries.

Funding Support: British Columbia Wine Grape Council, Canadian Grapevine Certification Network, Agriculture and Agri-Food Canada

#### Phenological Shoot Autonomy in Grapevines

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The common viticulture practice called green thinning, performed to improve uniformity of ripeness, is based on an underlying assumption that shoots on the same grapevine are phenologically autonomous. There is no empirical evidence to support this assumption. The objective of this study was to test this assumption. The theory of phenological shoot autonomy in grapevines was tested over two seasons in two different Merlot vineyards located in Napa Valley. The theory postulates that grapevines can not synchronize the phenological development of all their shoots. A trial was established comparing early pruning (EP) versus late pruning (LP) of spurs on separate vines (SV), or applied to the same vine in two forms: opposite cordons on the same vine (OC) or alternate spurs on the same vine (AS). Phenological stages were followed on shoots from one vine per replicate for each treatment combination using an updated Eichhorn-Lorenz scale from budbreak to end of veraison. The ordinal logistic regression analysis of the median phenological stages in both seasons revealed a significant effect of pruning date, but not vine level, at any time throughout either season, which supports the hypothesis of shoot autonomy. Budbreak was delayed by 10 days the first season and between seven and nine days the second season with late pruning. Veraison was delayed six to seven days each season with late pruning on both separate vines or same-vine treatments. Fruit maturity sampling also showed a significantly lower Brix in LP vines for all vine treatments and no interaction between pruning date and vine level in both seasons. There was no significant interaction between pruning date and vine level on phenology or Brix, which would have indicated that vines could synchronize



2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

phenological development. Overall, the results support the phenological shoot autonomy theory in grapevines.

Funding Support: Treasury Wine Estates

#### Integration of Vermicompost and Vermicompost Tea for Soil and Plant Health Management in Semiarid Vineyards

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There is increasing interest among winegrape growers in use of vermicompost (VC) and vermicompost tea (VCT). VC has fewer pathogens and higher nutrient concentrations than traditional compost. Studies on use of VC in vineyards have reported that VC can increase grape yield, supply high amounts of nutrients, enhance arbuscular mycorrhizae fungi colonization, and suppress populations of parasitic nematodes. The suppression of powdery mildew and botrytis bunch rot pathogens of grapevine with different biology and epidemiology by VCT indicates the potential for its use as an integrated disease management strategy in vineyards. The recent detection and quantitation of cytokinins in VCT provided direct evidence to explain the growth efficacy of applying VCT to enhance plant growth and development. When soil-applied, VCT has been observed to stimulate health and growth of plants, particularly roots, and to enhance diversity and activity of soil microbial communities, including suppression of parasitic nematodes. Fungi isolated from VCT have shown to be effective for their phosphate-solubilizing potential and antagonistic activities. First year yield and yield quality results from VC and VCT trials on Chardonnay in the Okanagan Valley, BC, Canada, will be presented. Interim data from the VC trial showed no significant effect on yield or yield quality parameters; however, petiole N at veraison increased significantly by 0.65, 0.80, 0.96, and 1.07% with VC application rates of 0 (fertilized control), 15, 30, and 45 tons fresh weight basis/ha row, respectively. Preliminary data showed increased yield (14%), average numbers of clusters (7.3%), and berry size (2.7%) with application of VCT (five applications at E-L growth stages of 15, 19, 23, 28, and 31; 1:25 compost to water ratio; 800 L/ha spray rate) compared with the control (distilled water).

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

#### Improvement of Sugar Accumulation Using Double-Variety Grafting under Mesic Conditions

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In Japan, winegrapes do not experience sufficient soil water stress after veraison because of hot temperatures and precipitation during the growing season. As a result, berries swell with water, and sugar content does not rise sufficiently. Fruit quality is particularly poor in red winegrapes. In general, yield limitation is useful to improve fruit quality, although it's not practical in small Japanese vineyards because of low income. If different varieties with different harvest timings could be mixed in the same vine, sugar would be loaded from the branch left after the early harvest to the late variety fruit, and fruit quality could be improved. Therefore, we conceived an improved grafting method that does not reduce yield. Early-ripening Chardonnay shoots were grafted onto already-cultivated Merlot vines, and one year later, the double-variety grafted vines were

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

compared with control vines composed only of Merlot. Of interest was the influence on fruit quality of sugar loading to black grapes and water stress due to transpiration from white grape shoots after Chardonnay harvesting. The quality of white grapes was not improved, but black grapes did not show noticeable swelling and Brix rose significantly. Black grapes also showed shedding of flowers slightly. Although this result means that the yield of Merlot vines grafted with Chardonnay would be decreased compared with the control group, it is thought that diseases will be less likely to occur by not overcrowding bunches during a rainy growing period. This approach can be also applied to improving viticulture in other humid regions. These studies will represent important values for the competitiveness and future sustainability of winemaking in Japan.

Funding Support: the Project of the NARO Bio-oriented Technology Research Advancement Institution (Integration research for agriculture and interdisciplinary fields)

#### Effect of Vineyard Site and Wine Age on the Aromatic Profile of Pinot noir Wines

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Volatile compounds in wine originate from grape components, microbiome, and yeast metabolism and autolysis, and are modified during aging due to chemical transformations and interactions. Using minimal sources of variation and having grapes harvested from vines planted on the same combination of scion and rootstock, this study investigates the impact of vineyard sites in the aromatic profile of Pinot noir wines. In 2015, grapes harvested at 23 to 25 Brix from 10 sites in California and two in Oregon were delivered to the UC Davis Teaching and Research Winery and processed according to a consistent protocol. Musts were fermented in quadruplicate, targeting the same ratio of berry/juice across fermentation replicates. After secondary fermentation, the wines were racked into stainless kegs (two/vineyard site). Samples were taken at the age of three months (from kegs) and after bottling at the ages of eight and 20 months (from screwcap bottles). Samples were analyzed in triplicate and 39 compounds were measured using headspace solid-phase microextraction (HS-SPME) gas chromatography-mass spectrometry (GC-MS). Data were analyzed using analysis of variance to determine the effect of vineyard site and wine age. Sites within the same AVA clustered together. The aging of the wines did not impact the relative presence of important compounds or the separation by AVA, which demonstrate the unique characteristics of the specific sites and how aged wines tended to remain different from each other with time. Wines from the Santa Rita Hills, Russian River Valley, and Sonoma County AVAs were found to be more similar among themselves, while one of the wines from Oregon was different from any other wine. Comparisons of the sensory perception and chemical composition of these wines will be made in the future, along with comparison of the volatile composition of wines made across different growing seasons.

Funding Support: Jackson Family Wines

WED/THUR POSTER ABSTRACTS



#### Impact of Cluster Thinning on Marketabilty Attributes of Table Grapes Grown in a High Tunnel System

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Production of table grapes (Vitus vinifera) is limited in the southeastern United States, but it could be possible using high tunnel systems. The impact of cluster thinning on marketability attributes of table grapes in high tunnel systems was evaluated. Table grapes were grown on a Geneva double curtain trellis in a high tunnel system at the University of Arkansas Agricultural Experiment Station, Fayetteville (cold hardiness zone 6b). Two cluster-thinning treatments (none and pea-sized berries) were applied to vines of three cultivars (Faith, Gratitude, and Jupiter). The grapes were hand-harvested in July 2018. Two clusters per 0.9 kg-clamshell were evaluated in triplicate for composition (soluble solids, pH, and titratable acidity) at harvest (day 0) and marketability attributes (shatter, decay, and weight loss) during storage (0, 7, 14, and 21 days) at 2°C. At harvest, grapes with no thinning had higher soluble solids (18.03%) and pH (3.72) than thinned grapes (17.10% and 3.61, respectively), but thinning did not impact titratable acidity (0.54%). Faith had higher soluble solids than Jupiter or Gratitude, but Gratitude had a lower pH and higher titratable acidity than Faith or Jupiter. During storage, Jupiter had more shatter (7.21%) than Gratitude (4.78%) or Faith (3.62%), and shatter increased from 2.98% to 7.41% after 21 days. Faith with no thinning (9.96%) had more decay than pea-sized thinning and both thinning treatments for Gratitude and Jupiter (2.33 to 4.64%). As storage increased, weight loss increased for all cultivars. Faith at 21 d storage had the most weight loss (5.11%). Faith with no cluster thinning had more weight loss (2.84%) than the other cultivars and thinning treatments. At harvest, cultivars differed in composition attributes, and cluster thinning lowered soluble solids and pH of grapes. During storage, weight loss and shatter increased for all cultivars, but variations in marketability attributes were cultivar-specific.

Funding Support: Southern Sustainable Agriculture Research and Education Grant, United States Department of Agriculture (RD309-137/S001415).

#### Leaf Area to Crop Load Ratio Only Affects Berry Ripening after Two Years of Treatments

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The concept of vine balance is used widely in vineyard management to optimize yields and ripening stage at harvest. However, most of our knowledge in this area is based on intuition or on indirect observations. We performed a two-year trial (2017 and 2018), applying three levels of defoliation (keeping 100, 66, or 33% of the leaves) and crop load reductions (keeping 100, 66, or 33% of the clusters) in a factorial design (3 Đ 3) on Cabernet Sauvignon (FPS08) grafted onto 110R. In the first year, must soluble solids were greatly affected by reduced canopy density, and the time to pass the 25 Brix milestone took nearly six weeks longer with 33% leaves than with 100% leaves treatment. Contrarily, crop load did not affect soluble solids, and thus, vines with three times less fruit reached 25 Brix on the same date. In the second year, soluble solids were responsive to both factors and were correlated tightly with the leaf area/fruit ratio. This finding highlights the capacity of healthy vines to maintain ripening rates under adverse conditions for one season. Vines also had remarkable mechanisms to compensate the

## Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

lack of vine balance. For instance, in comparisons among treatments with the same cluster number, vines with lower leaf area had lower yield mediated by reduced berry size. Interestingly, vines with 33% leaves had a lower root mass and dormant pruning mass. These results provide hints for vineyard management affecting ripening, yields, berry size, vigor, and root growth over the years. On a third year, carryover effects on yields and ripening speed will be analyzed to find the most sustainable leaf area/fruit ratio for the long term.

Funding Support: American Vineyard Foundation

## Blue Light-Emitting Diode (LED) Irradiation Accelerates Coloring of Winegrapes

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It has been reported that blue light irradiation of grape bunches promotes coloring in grape skin, in the same way as ultraviolet irradiation. Little is known about the detailed timing of blue light irradiation. Therefore, we investigated coloring in Merlot, a winegrape cultivar, associated with the timing of blue light-emitting diode (LED) irradiation. Grape bunches were irradiated with blue LED set 30 cm below the bunches for six hours daily from the beginning of veraison to harvest. Fruit quality, anthocyanin content, and anthocyanin composition were investigated. Total anthocyanin content increased more rapidly in irradiated grape skin than in untreated grape skin. As regards anthocyanin composition at the time of increase, the levels of peonidin-3-glucoside, petunidin-3glucoside, malvidin-3-glucoside, and two kinds of malvidin-based anthocyanins increased in irradiated grape skin relative to untreated grape skin. Total soluble solids in blue light-irradiated grape juice were the same as those in untreated grape juice at harvest, but total acidity content was greater in the irradiated grape juice. Thus, anthocyanin content in grape skin was increased and grape berry maturation was promoted by the blue LED irradiation. As harvest time approached, total anthocyanin content and the levels of cyanidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside, and two kinds of malvidin-based anthocyanins in irradiated grape skin decreased to match those in untreated grape skin. Supplemental blue LED irradiation at veraison influences anthocyanin biosynthesis and accelerates coloring and maturation of winegrapes.

Funding Support: Meijo University Research Branding Project

#### Preventing Smoke Taint in the Vineyard

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Research has demonstrated that the waxy cuticular layer of the grape is involved in the uptake of the presumptive causative agents of smoke taint (i.e., volatile phenols, VPs) during smoke exposure, but it also serves a protective role by insulating grapes from environmental factors like exogenous VPs. Given this dominant ingress mechanism (minor foliar uptake has also been demonstrated), one approach that has not been explored extensively in the literature is using protective sprays to limit uptake of VPs into the berry during smoke exposure. Accordingly, this study evaluated several commercial products with spray-based application for their ability to limit uptake of



VPs. More specifically, a wax-based biofilm and two oil-based antifungal sprays were evaluated for their ability to insulate grapes from the effects of wildland fire smoke. While none of these products were designed to protect grapes against smoke taint, investigating their use in this regard is an important research avenue with the potential to provide a broadly applicable solution to mitigate smoke taint in the vineyard. Our results indicate that the wax-based biofilm, applied one week before smoke exposure, reduced the concentration of free and bound VP smoke-taint marker compounds in Pinot noir grapes by up to 400%, compared to grapes that did not receive the spray treatment. The oil-based sprays did not appreciably change the detected concentrations of smoke taint marker compounds in Pinot noir grapes, suggesting that their use to control smoke-associated fungal issues is unlikely to make smoke taint worse. As a preliminary study, these data strongly suggest a path forward that will provide viticulturalists with a viable tool to help protect their grapes in the face of more frequent and severe wildland fire seasons.

Funding Support: Natural Sciences and Engineering Research Council, Canadian Foundation for Innovation

## Fire, Water, and Wine: Effects of the North Bay Wildfires on Napa Valley Vineyard Soil Carbon and Sulfur Losses

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In October 2017, the North Bay wildfires swept through hillsides of California's Napa and Sonoma winegrowing regions. We asked how the wildfires affected storage and transport of organic carbon (C), an important soil stabilizer and energy source in stream nutrient cycles, and sulfur (S), which is applied to vineyards to combat powdery mildew. Prior research showed that S transport in vineyards is controlled by dormant season rainstorm events. Thus, we designed a laboratory soil leaching experiment to measure rain event-based and seasonal losses of S and C from soils. We collected soil cores (0 to 10 cm) from three site types: nonburned grassland, nonburned vineyard, and burned vineyard. We simulated 10 rain events from the 2017 to 2018 wet season and collected soil leachates. We also sampled streams throughout the Napa River watershed during one rain event to measure post-fire watershed-scale C and S fluxes. Soil leachates and stream samples were analyzed for S species and dissolved organic carbon (DOC) concentrations. We found that while burned and unburned vineyard soils leached more S than grassland soils, unburned soils leached less DOC than burned soils. Burned vineyard soils leached on average three times less S, but 2.5 times more DOC than unburned vineyard soils. For all soil cores, leachates from rain events over 50 mm had ~3 times greater DOC and S concentrations than smaller events. In contrast to what we expected, DOC concentrations of stream samples from burned and unburned sampling locations were not significantly different. Streams draining vineyard-dominated landscapes, however, had significantly more S than burned or unburned forest landscapes, suggesting that vineyard soils export more S than grasslands, likely from fungicidal applications during the growing season. The increase in DOC losses from burned vineyard soils suggests that winegrowers may need to amend soils with organic carbon (e.g., cover crops) when replanting burned vineyards.

Funding Support: National Science Foundation, University of Colorado Boulder Environmental Studies Program, University of Colorado Boulder Center for Water, Earth Science, and Technology

#### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

#### Conversion to Mechanical Pruning Maintains Fruit Composition while Reducing Labor Costs in Merlot Grape

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A field study was conducted for three consecutive seasons in the hot climate of central California to assess the performance of Merlot grapevine (Vitis vinifera L.) grafted onto Freedom (Fresno 1613-59 Đ Dog Ridge 5, a 27% V. vinifera hybrid) during training system conversion to facilitate mechanization. The traditional head-trained and cane-pruned (CP) system was either retained or converted either to a bi-lateral cordon-trained, spur-pruned California sprawl training system (HP), or to a bi-lateral cordon-trained, mechanically box-pruned single high-wire sprawling system (SHMP). After the conversion, SHMP sustained greater yield with more clusters/vine and smaller berries without affecting the canopy microclimate. This was due to more nodes retained after dormant pruning. The SHMP canopies filled allotted canopy space earlier than CP and HP, based on photosynthetically active radiation transmitted through the canopies, populating the space allotted/vine and favoring greater production efficiency. There were no adverse effects of training systems on berry composition or flavonoid concentration during or after conversion to mechanical management. However, an experimental year effect was obvious on the anthocyanin composition of Merlot berries, with increasing tri-hydroxylated (i.e., delphinidin-based) anthocyanins in the latter years of the experiment. Our results also provided evidence that earlier canopy growth, coupled with sufficient reproductive compensating responses, allowed for increased yields while reaching commercial maturity without a decline in anthocyanin content in SHMP. Converting CP to SHMP reduced labor operations costs by 90%. Furthermore, SHMP had greater gross revenue and resulting greater net income/acre even when the conversion year was taken into account. Therefore, SHMP is recommended for growers within the hot climate of the central San Joaquin Valley (SJV) as a means to maintain productivity of vineyards while not sacrificing berry composition at the farm gate.

Funding Support: American Vineyard Foundation

#### Potential Use of High-Resolution, Remotely-Sensed Images from Unmanned Aerial Vehicles in Vineyard Weed Management

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In recent years, remote-sensing technology is being explored as a management tool in agricultural cropping systems, including vineyards. However, the technology has not been explored adequately for weed management. Early identification of critical zones by drones would enable site-specific weed management and avoid broadcast application of post-emergence herbicides in vine rows. The experiment was conducted in a winegrape vineyard in Napa County, where interrows were disked while under-vine weeds were mechanically managed multiple times during the growing season. Aerial images were obtained by a drone equipped with a multispectral camera. Two flight heights were tested: 30 m and 10 m above the vineyard floor with a resolution of 0.5 cm/px and 1 cm/px, respectively. Based on field NDVI (normalized difference vegetation index), two management zones were identified and data on percent weed coverage and dry biomass were taken at three different locations. Species were also identified on site. For mapping and visualization, image mosaics were obtained with DroneDeploy, and



data were analyzed and modeled. Results showed that areas of weed presence could be identified successfully by the drone images. Vine vigor did not correlate with weed density or biomass. The image resolution was good enough to identify several weed species. It can be concluded that remote sensing with drones can aid in site-specific weed management in vineyards.

Funding Support: California State University

#### Leveraging High Resolution Hyperspectral and Thermal UAV Imageries for Characterizing Diurnal Grapevine Physiology

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Efficient methods to monitor vine physiological response to water stress help improve vine productivity and clarify their physiology. Developments in unmanned aerial vehicles (UAV) and sensor technology have provided images at very high spectral, spatial, and temporal resolutions. However, studies focusing on the characterization of vine physiology using high-resolution UAV imagery are lacking. This study aims to (i) develop novel approaches to estimate vine physiological conditions using high resolution (<10 cm) hyperspectral and thermal imagery, and (ii) investigate the potential of a new zonalweighted method to better estimate in-situ water potential, stomatal conductance, fluorescence, and photosynthetic measurements. Diurnal camera flights over an experimental vineyard in Mount Vernon, MO, took place over two years during different vine growth stages. The vineyard was designed to investigate the effects of irrigation levels (0, 50, and 100%) on Chambourcin grown ungrafted and grafted to 1103 Paulsen, 3309 Couderc, and SO4 rootstocks. Midday and afternoon in situ measurements indicated greater stress in the ungrafted vines than on vines grafted on SO4. Extracted pure vine canopy from the UAV imageries was divided into three different zones (sunlit, nadir, and shaded). Then, common remote-sensing stress indicators such as photochemical reflectance index, sun-induced chlorophyll fluorescence, and canopy water stress index were extracted. Using the strength of the correlation between the remote sensing indicators and the in situ measurements as weight factors, weighted remote-sensing indicators from different canopy zones were combined and their correlation with the in situ measurements was explored. The preliminary results showed that the correlation from different canopy zones varied, with the sunlit zone providing the best estimate in most instances. The weighted stress indicators always significantly improved estimation performance.

Funding Support: Missouri Grape and Wine Institute at the University of Missouri-Columbia, National Science Foundation Plant Genome Research Program 1546869

#### Testing Nematode-Resistant Rootstocks for San Joaquin Valley Viticulture

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Plant parasitic nematodes may extensively damage grapevine roots that are not resistant to them, especially in vineyards with sandy soils, as are common in the San Joaquin Valley of California. Fumigation is an effective control measure; however, regulations have restricted the use of fumigants in California, heightening the importance of nematode-resistant rootstocks. The development of better nematode-resistant rootstocks is an ongoing effort. Documenting the viticultural performance of

## Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

scions grafted to rootstock selections is critically important for proper selection and commercial acceptance of rootstocks and should be part of the process in selecting new material for potential release. Therefore, the performance of Malbec winegrapes on newer nematode-resistant rootstocks (RS-3, RS-9, GRN2, GRN3, and GRN4), along with 1103P as a control, were planted in a replicated trial in a commercial vineyard in Merced County. Each replicate is a full row of 388 plants with four replicates/rootstock. Data from the second year of growth suggest that the various rootstocks do not affect the timing of phenological events, and so far, all vines appear to have sustained similar growth, as measured by trunk diameter. Petiole samples indicate that the various rootstocks differ with respect to the uptake of potassium and many different micronutrients at both bloom and veraison. However, neither nitrogen nor phosphorus differed according to rootstock at bloom or veraison. This ongoing trial will provide useful data for growers needing nematode-resistant rootstocks.

Funding Support: American Vineyard Foundation

## Rapid Screening for Salt-Stress Tolerance Through Chloride-Ion Accumulation in Leaves of Wild *Vitis* spp. Rootstocks

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Increasing ambient temperatures and changes in precipitation have fueled growing concerns of salt accumulation in agricultural soils. Salt stress in grapevine (Vitis spp.) is primarily attributed to chloride anion accumulation in aboveground tissues, rapidly reaching toxic concentrations and promoting detrimental responses from the plant. Sodium tends to be excluded by grape roots in favor of potassium and does not accumulate to toxic levels before chloride toxicity begins. However, chloride toxicity in the scion may be minimized by using rootstocks with high chloride exclusion. These rootstocks may reduce long-distance transport of chloride anions to actively growing tissues aboveground. Novel material from the southwestern regions of the United States was screened for chloride ion accumulation in laminae and petiole tissues under controlled conditions in a greenhouse setting. Potted vines of each accession were grown for 28 days without salt exposure, followed by 28 days of irrigation with dissolved salt water at 75 mM NaCl concentration. Four rootstocks with known response were selected as controls and were irrigated only with Hoagland's solution throughout the trial. Tissue samples were then collected and dried, and chloride concentrations were quantified using silver-ion titration methods. A wide range of tissue-chloride concentrations was evident in this trial, ranging from 5mM Cl<sup>-</sup> to >1 mM Cl<sup>-</sup> in some cases. Accessions from arid regions had lower chloride ion concentrations in the measured tissues. The individual that displayed the greatest chloride exclusion was an accession of Vitis acerifolia 9018, previously tested for nematode resistance. Further work will include field trials of accessions identified as having the greatest chloride exclusion potential and assessments of root chloride sequestration and xylem chloride concentrations in vivo.

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#### **Effects of Rootstocks on Merlot Winter Hardiness**

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The hardiness of roots and buds of young own-rooted Merlot vines and Merlot grafted on Riparia Gloire, SO4, 3309C, 101-14, 5C, Schwarzmann, 110R, and Ramsey rootstocks, was determined in the Okanagan Valley, British Columbia. In July, the four-month-old vines were field-planted in a randomized block design, with six vines/plot to allow for repeated destructive sampling during the fall and winter. At each sampling during November and December, a single vine/plot was harvested, and the hardiness of its roots and buds was determined using differential thermal analysis. All rootstocks except 110R had hardier roots than Merlot's own roots. Rootstocks varied in root hardiness, with 101-14 and 110R being the most and least hardy, respectively. Root hardiness increased over the sampling period, indicating that roots gain hardiness as temperatures decline in the fall. Bud hardiness increased earlier and reached its maximum sooner in Merlot grafted onto rootstocks than in own-rooted vines. By mid-December, buds had reached maximum hardiness, and there was no difference among grafted and own-rooted vines.

Funding Support: Canadian Grapevine Certification Network and Agriculture and Agri-Food Canada

#### Effect of Agar on Growth of Roots of Six Grapevine Rootstocks

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Root morphology plays a critical role in the response of plants to water availability. However, the analysis of root architecture can be very difficult. Our lab has tested a variety of methods to characterize the rooting patterns of commercial rootstocks and hybrids to develop rapid and reliable phenotyping methods for use in our breeding programs. We analyzed the response of several rootstocks growing in vitro under increasing concentrations of gelling agent. Apices from micro-plants of rootstocks Ramsey, 1103P, 101-14Mgt, and Riparia Gloire de Montpellier were subcultured into clear Falcon tubes containing 30 mL Nitsch and Nitsch medium supplemented with 20 g/L sucrose, 5 Qg/L NAA, and 5 Qg/L biotin. The medium was solidified with 1, 5, or 10 g/L Gelzan (PhytoTechnology Labs). Each treatment was replicated five times. The time required for roots to reach the base of the tube and root fresh weight after 10 weeks was recorded. Only one rep of Ramsey reached the bottom of the tube at 1 g/L, while all grew to the bottom, except Riparia, at 10 g/L. At 5 g/L, two to four reps grew all the way down. Roots of 101-14 grew as fast and deep as 1103P and Ramsey but were much less fibrous. ANOVA analysis and Fisher test of root fresh weight showed statistical differences among cultivars (1103P higher than the rest) and agar concentration (1 g/L lower than the rest). Three-dimensional root phenotyping could allow better characterization of the differences observed in this simple and quick assay.

Funding Support: California Grape Rootstock Improvement Commission

#### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

#### Enology and Viticulture Research Report Posters—CONTINUED

## Using Metabolomic Analysis to Characterize Volatile Compounds in Cold-Hardy Hybrid Grapes in Minnesota

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Flavor is one of the most important attributes of fruit crops and is a major factor in consumer acceptance and repeat purchase. However, the measurement of this trait is often limited due to its subjectivity. The taste of a "pleasant" or "unpleasant" flavor is the result of a specific, or group of specific, volatile compounds. The concentrations of these compounds depend on the grape variety, management practices, and environment where the grapevines are grown. Hybrid grapes produce flavor compounds that taste different from the traditional Vitis vinifera species, affecting sensory and quality perceptions. The objective of this study was to identify the compounds present in grapes from a segregating progeny (n = 25) derived from a cross between Itasca and MN1250. Using liquid-liquid extraction and gas chromatography-mass spectrometry (GC-MS) methods, volatile compounds were identified from each seedling tested. Seeking to evaluate the perception of these compounds in each sample, sensory testing was conducted with five panelists from the University of Minnesota's Horticulture Research Center. Panelists evaluated randomized, duplicated samples from 25 seedlings and rated important sensory attributes of grapes such as sourness, sweetness, and flavor (muscat, candy, labrusca, and riparia/hybrid) using a 0 to 20 scale. Analysis of the scores revealed that panelists rated flavor intensity of the berries higher than aroma intensity. Panelists identified herbaceous (i.e., green plants, green apple, and grass) and labrusca (i.e., candy, fruity, and Concord) as the dominant flavors present in the samples. Based on the sensory test results and the >1000 volatile compounds identified by GC-MS analysis, we identified compounds that were presented in each flavor category. The results of combining GC-MS and taste panels to identify specific flavor compounds will be helpful for future work developing an association between these compounds and quantitative trait loci (QTL) related to flavor compound production.

Funding Support: Minnesota Department of Agriculture/University of Minnesota

## 24-Epibrassinolide-Induced Foliar Defenses against Downy Mildew in Cabernet Sauvignon and Ecolly

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Downy mildew, caused by *Plasmopara viticola*, is a fungal disease that destructively decreases grape quality and yield. Brassinosteroid has emerged as an important phytohormone that regulates plant defense and is involved in induced resistance. The goals of our study were to evaluate the efficacy of exogenous brassinosteroid against infection by *P. viticola*, to investigate the responses of endogenous plant hormones, and to monitor the relative impact of brassinosteroid treatment on plant defense genes. The effect of 24-epibrassinolide (EBR) on downy mildew was studied on detached leaves of Cabernet Sauvignon (*Vitis vinifera*) and Ecolly (*Vitis vinifera*). Application of 0.5 mg/L EBR significantly reduced disease severity and mycelium growth of downy mildew on both Cabernet Sauvignon and Ecolly. EBR treatment enhanced the activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) in both cultivars, indicating increased tolerance to oxidative stress. The amounts of endogenous abscisic acid, salicylic acid (SA), and jasmonic acid were increased significantly in EBR-treated grape leaves within five days postinoculation.


2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

RT-PCR analysis showed EBR treatment induced expression of SA-mediated regulatory genes and three pathogenesis-related genes: *PAD4, NPR1,* and *PR1.* Our results provide evidence that EBR application contributes toward activation of foliar defense responses in both Cabernet Sauvignon and Ecolly.

Funding Support: National Natural Science Foundation of China (31772258)

# Early Manual and Mechanical Leaf Removal as a Strategy to Improve Ripening and Lower Cluster Rot Disease in Pinot grigio

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Removal of basal leaves early in grapevine vegetative and reproductive development is a tool widely used to decrease fruit set. In turn, this technique controls yields and modifies cluster architecture, subsequently lowering cluster rot severity and improving fruit quality. However, the phenological stages near bloom recommended for application of this practice are short, and the considerable time required for implementation limits its use by grapegrowers. Efficient mechanization can potentially mitigate these issues but has not yet been compared with manual application in a cool, humid growing region where cluster rot is the major limitation for yield and fruit quality. The goal of this study was to compare mechanical leaf removal (ME) with manual (MA) removal of six leaves at prebloom (PB) and after-bloom (AB) phenological stages over two seasons in Pinot grigio, a tight-clustered cultivar. Results indicate that fruit set and cluster compactness were only reduced in both PB treatments. While the loss of fruit due to grey mold and sour rot was partially lowered in PB-ME and AB-MA, only PB-MA had a consistent reduction, indicating the importance of a clear fruit zone in addition to decreased cluster compactness to significantly mitigate cluster rot diseases. Interestingly, both prebloom treatments enhanced fruit quality, likely driven by a similar reduction in fruit set. The results suggest that the implementation of mechanical leaf removal at the prebloom phenological stage may be used to reduce cluster rot in tight-cluster cultivars and enhance ripening, regardless of cluster morphology. This information provides a single approach to alleviate two prominent issues facing seasonal management strategies in cool climate viticulture.

Funding Support: Michigan Grape and Wine Industry Council and Project GREEEN

# Characterization of Pierce's Disease Resistance in b41-13, an Accession Collected from Tamaulipas, Mexico

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Identification of novel sources of Pierce's disease (PD) resistance and tagging genomic regions to facilitate marker-assisted breeding is one of the key goals of the grape breeding program at UC Davis. Previously, we identified strong PD resistance in an accession, b41-13, from Tamaulipas, Mexico, which differed from a previously characterized PD resistance locus *PdR1*. The accession b41-13 appears to be a hybrid of *V. arizonica, V. candicans*, and *V. champinii* and provides the opportunity to expand the genetic base of PD resistance. An F1 breeding population of 295 seedling plants was developed by crossing the susceptible *V. vinifera* (F2-35) with b41-13. Replicated seedling plants were propagated for greenhouse screening, and disease evaluations were completed on all F1 plants using inoculated and un-inoculated reference controls. A total of 600 SSR markers were tested on a small set of parents and progeny to

### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

identify polymorphic markers; 53% of the markers were polymorphic and were added to the entire set of 295 seedling plants to develop a framework map. Parental and consensus framework genetic maps will be developed with JoinMap V.4.0. Quantitative trait analysis will be carried out using the Map QTL program to identify genomic regions with potential PD resistance loci. Results will be discussed in terms of a new genomic region for PD resistance and development of markers that could be used for markerassisted breeding.

We gratefully acknowledge research funding from the CDFA Pierce's Disease Board and the Louise Rossi Endowed Chair in Viticulture.

# Comparative Sequence Analysis of the PD Resistance Locus *PdR1* in Two Resistant Accessions: b43-17 and b40-14

**Summaira Riaz**,\* Cecilia Agüero, Rong Hu, and M. Andrew Walker \*UC Davis, Dept. Viticulture and Enology, Davis, CA 95616 (snriaz@ucdavis.edu)

Genetic mapping can identify genomic regions that harbor resistance genes. Previously, we identified the PdR1 locus at the same genomic position on chromosome 14 in two accessions, b43-17 and b40-14. In this study, we developed a physical map of the PD resistance locus for both accessions, carried out comparative sequence analysis between the two resistant accessions, and compared them to the reference grape genome sequences of PN40024 and Cabernet Sauvignon. Bacterial artificial chromosome (BAC) libraries were developed using genomic DNA that provided eight to 10 X genomic coverage, with an average insert size of 140 to 160 kb. Libraries were screened with closely linked markers to identify corresponding BAC clones that had overlap with each other and represented the genomic region of the PdR1 locus. For the accession b43-17, five BAC clones were sequenced using the PAC BIO RSII sequencing approach. Four of these BAC clones represented the PdR1b locus and spanned a 604 kb region; BAC clone H43-I23 represented the PdR1a locus and spanned 206 kb. Complete homology was observed between the overlapping BAC clone sequences that reflect two different haplotypes of the *PdR1* locus. For the accession b40-14, four BAC clones were selected from a set of 30 that represented the PdR1c region. The assembled region consisted of 426 kb of physical sequence. Results will be discussed in terms of similarities and differences in the genomic regions of the resistant and susceptible sequences, and how they function and evolved.

Funding Support: We gratefully acknowledge research funding from the CDFA Pierce's Disease Board and the Louise Rossi Endowed Chair in Viticulture.

## *Drosophila* Egg and Larval Stages Cause Sour Rot Symptoms at Same Rate as Adults

#### Patrick Kenney\* and Megan Hall

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Sour rot in *Vitis* spp. is a late-season disease in viticultural regions worldwide, described by the onset of berry skin oxidation, oozing of berry pulp, and the smell of acetic acid, all in the presence of fruit flies (*Drosophila* spp.). Healthy berries of *Vitis* interspecific hybrid cv. Chambourcin were surface-sterilized, wounded, and co-inoculated with *Metschnikowia pulcherrima* and *Gluconobacter cerinus*, known causal organisms of sour rot. Inoculated berries were exposed to one of three developmental stages of axenic *D. melanogaster*: eggs, 24 hr-old larvae, or adults, to compare the ability of various life stages to cause symptoms and to determine the rate of symptom development.



Symptoms were rated daily on a previously-defined 0 to 4 scale. Over nine days, disease progression occurred at the same rate and reached the highest rating level (4) in all life stages. This suggests that egg and larval stages, not just adults, can play a role in sour rot development. In a separate experiment during harvest 2018, clusters of varying *Vitis* cultivars displaying sour rot symptoms were collected from commercial vineyards throughout Missouri and placed in plastic cups. At harvest and one week and two weeks postharvest, each cluster was rated for sour rot severity (% of cluster affected) and the number of adult fruit flies reared from each cluster was counted. Adults were removed from clusters at harvest so as to determine the effect of other life stages on symptom progression. Within two weeks, a mean of 92 fruit flies had emerged and severity had increased from 30 to 80%, indicating that eggs and larvae were present in the clusters at harvest, and a combination of life stages was likely responsible for the increase in severity. Both experiments demonstrate that *Drosophila* eggs and larvae, not just adults, play roles in sour rot development, which has major implications for disease management strategies.

Funding Support: Missouri Wine and Grape Board Research Committee Grant Millikan Endowment Fund

#### Impacts of Viral Diseases in Field-Grafted Vineyards in Washington State

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Vineyards are traditionally planted in different grapevine-growing regions with grafted vines, consisting of vinifera scion cultivars grafted onto the root system of non-vinifera rootstocks. In contrast, vineyards in Washington State are widely planted with ownrooted vines. In recent years, however, many growers are adopting field-grafting to quickly replace existing vinifera cultivars with other preferred cultivars to keep up with trending market demand. Although impacts of viral diseases have been examined in grafted and own-rooted vinifera cultivars, similar studies were not conducted in viniferato-vinifera field-grafted vineyards. In this study, impacts of viral diseases on fruit yield and berry quality attributes were measured in vineyard blocks field-grafted with three red-fruited cultivars (Cabernet Sauvignon, Merlot, and Syrah) onto Riesling trunks. In Cabernet Sauvignon and Merlot blocks, data on fruit yield and berry quality was collected at the time of commercial harvest from symptomatic and non-symptomatic vines tested positive and negative, respectively, for Grapevine leafroll-associated virus 3 (GLRaV-3). In the Syrah block, data on fruit yield and berry quality was collected at the time of commercial harvest from symptomatic and non-symptomatic vines tested positive and negative, respectively, for Grapevine red blotch virus (GRBV). The results from 2017 and 2018 seasons indicated significant impacts of GLRaV-3 and GRBV on fruit yield, soluble solids, and berry anthocyanins in vineyard blocks field-grafted with Cabernet Sauvignon, Merlot, and Syrah. However, impacts on fruit yield and quality were variable between cultivars and the type of virus. Impacts of GLRaV-3 and GRBV on grape juice pH were not as pronounced as impacts on fruit yield, berry sugars, and anthocyanins, although higher levels of grape juice TA was observed in all cultivars. These results provided insight as to how two distinct viruses affect vine health and fruit quality in field-grafted vineyards.

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119

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

## Search for New Sources of Resistance to Pierce's Disease: Characterization of the PD Resistant Accession b46-43

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The causal agent of Pierce's disease (PD) is the xylem-limited bacterium Xylella fastidiosa. The bacteria obstruct the xylem of infected vines, hindering water transport. Vitis vinifera varieties have different levels of susceptibility, but all varieties die within five years of infection. A number of PD control strategies have been proposed, but none offer a long-term sustainable strategy. Breeding efforts in the Walker Lab at UC Davis focus on developing resistant grapevines by finding genetic sources of PD resistance. Previous research identified a PD resistance gene (PdR1) in a wild grapevine from Mexico, which has been used to breed PD-resistant winegrapes. The discovery of PDresistant accessions from Mexico and the southern United States prompted screening of wild grapevines from these regions. The objective of this study was to characterize PD resistance in the accession b46-43, collected in Big Bend, TX. Population diversity metrics suggested that b46-43 contains a unique form of PD resistance. We have evaluated 321 individuals from a population with b46-43 in its background. The progeny were inoculated with X. fastidiosa and evaluated with microsatellite markers. These data were used to identify a quantitative trait locus (QTL) on chromosome 14 and the possible presence of a minor locus on another chromosome. We are developing a framework genetic map to study the genetic basis of b46-43's resistance. This project will benefit the PD breeding program's goal of combining multiple forms of PD resistance to achieve durable and broad resistance to the disease.

Funding Support: CDFA PD/GWSS Board and CONACYT

Powdery Mildew Resistance Varies in Western United States Vitis Accessions

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*Vitis vinifera* grape cultivars are highly susceptible to grapevine powdery mildew, *Erysiphe necator*, a North American disease that has spread across all grapegrowing regions over the past 170 years. In this study, 170 wild grapevine accessions native to the western United States, from 18 *Vitis* spp., were tested to determine their susceptibility to powdery mildew. The highly susceptible *V. vinifera* winegrape Carignane was used as a control. All accessions were subjected to the same testing procedures. A vacuumoperated settling tower was used to inoculate young detached leaves to ensure uniform conidial deposition. A single isolate, the C-strain, was used for initial screening. The more resistant accessions were retested with another isolate, E1-101, to examine racespecific resistance. The most resistant accessions were found within the following species: *V. acerifolia*, *V. aestivalis*, *V. arizonica*, *V. berlandieri*, *V. candicans*, *V. cinerea*, and *V. doaniana*. However, responses within a given species were highly variable.

Funding Support: US AID and the Louise Rossi Endowed Chair funds



## Supplemental Vineyard Inputs May Partially Mitigate Negative Effects of Grapevine Red Blotch Disease in Pinot noir

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Grapevine red blotch disease (GRBD) poses an increasing threat to winegrape production by altering vine physiology and reducing fruit quality. While progress has been made in understanding GRBD pathology and epidemiology, few pragmatic horticultural strategies have been identified to manage disease effects in the field. Consequently, two field experiments were initiated in a commercial vineyard to determine the potential of cultural practices to mitigate negative effects in Grapevine Red Blotch Virus-infected (GRBV+) Pinot noir grapevines. In experiment A, vines received factorial combinations of grower control (GC) and supplemental (SUPP; 2x GC) irrigation (I) and fertilization (F). In experiment B, vines grafted to Couderc 3309 (3309C) or Riparia Gloire (RG) rootstocks received factorial combinations of GC and SUPP irrigation (I; 2x GC) and crop thinning (T; one cluster/shoot). Across both experiments, SUPP-I significantly reduced disease severity (% red leaves/vine) at harvest and increased yield over GC-I. SUPP-I reduced disease severity by 19.6% in experiment A, and by 15.2 and 10.0% in experiment B for 3309C and RG, respectively. Yields increased by 20 to 30% with SUPP-I across both experiments. SUPP-F or SUPP-T had no effects on disease severity, and SUPP-F had no effects on yield. There were no effects on total soluble solids (TSS) at harvest in either experiment, though SUPP-F and SUPP-T significantly increased berry pH. SUPP-F increased pH by 0.09 pH units in experiment A, and by 0.22 and 0.13 pH units in experiment B for 3309C and RG, respectively. The first year's data suggests that increasing irrigation can significantly reduce disease severity and increase yield with no significant reduction in TSS in GRBV+ Pinot noir grapevines. In contrast, supplemental fertilization and crop thinning did not affect disease severity and TSS, but did increase berry pH. Ultimately, increasing vineyard inputs may partially mitigate negative effects of GRBD.

Funding Support: Oregon Wine Board Oregon Department of Agriculture, Rogue Valley Winegrowers Association and the Oregon Wine Research Institute

# Occurrence of Economically Detrimental Viruses in Certified Nurseries and Commercial Vineyards in Washington State

**Nomatter Chingandu**, Sridhar Jarugula, Adilakshmi Movva, and Naidu Rayapati\* \*Washington State University, 24106 N. Bunn Rd, Irrigated Agriculture Research & Extension Center, Prosser, WA 99350-8694 (naidu.rayapati@wsu.edu)

As part of the clean plant campaign, a project was initiated to examine the sanitary status of registered grapevine mother blocks in Washington State nurseries. A composite sampling strategy was used to test leaf samples and dormant cane samples obtained from nurseries. Molecular diagnostic assays were used to detect *Grapevine leafroll-associated virus 3* (GLRAV-3) and *Grapevine red blotch virus* (GRBV), two viruses that are economically detrimental to vine health and grape quality. In 2018, 5572 composite samples collected from 27,003 vines belonging to 49 winegrape cultivars from two registered nurseries, 249 (0.92 %) tested positive for GLRAV-3 and none for GRBV. These results, together with the data from previous years, indicated the absence of GRBV and very low incidence of GLRAV-3 in grapevines maintained in registered mother blocks. Of 1391 samples belonging to 22 cultivars received from winegrape growers, 823 samples (59.16 %) were positive for GLRAV-3 and 63 samples (4.53 %) positive for GRBV. Additionally, 37 samples (2.66 %) from five cultivars received

WED/THUR POSTER ABSTRACTS

### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

from growers tested positive for both GLRaV-3 and GRBV, indicating co-infection of the two viruses. Together with previous data, these results indicated the occurrence of both GLRaV-3 and GRBV in grapevine samples obtained from grower vineyards, with GLRaV-3 being more common than GRBV. Efforts continue to test grapevines in registered mother blocks, so that growers can have virus-tested "clean" planting materials available from certified nurseries for planting new vineyards. In addition, commercial vineyards are monitored annually to document viruses and implement appropriate strategies for managing viral diseases in vineyards.

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### Outreach for California's Grapevine Certification Program and Area-Wide Management of Grapevine Virus Diseases

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Certified, disease-tested planting stock is a critical resource at the foundation of effective integrated management of grapevine virus diseases. The vectored spread of economically important grapevine viruses such as Grapevine leafroll-associated virus 3 (GLRaV-3) and Grapevine red blotch virus (GRBV) has challenged confidence in the California certification program, as registered nursery increase blocks are at risk for infection by these viruses. Vineyards newly established with certified stock can also become infected rapidly through vectored transmission of viruses present in neighboring blocks. Because vectors spread these viruses across property lines and transmission depends on infected vines in the landscape serving as virus reservoirs, coordinating management on local and regional levels is essential for long-term success. Several grower groups have self-organized to increase awareness among their membership and to organize coordinated management. Extension plays an important role in supporting such grassroots responses to complex, industry-wide challenges like grapevine virus disease management. In supporting these groups, we have taken lessons from how each group has responded according to its regional culture and opportunities, with lessons for how other groups may approach dealing with this important industrywide challenge and applications of coordinated management beyond virus diseases. We have also conducted a multi-year survey of grapevine viruses in young vineyards that confirms that GLRaV-3 and GRBV are the primary concerns in vineyards planted with certified stock.

Funding Support: Pierce's Disease/Glassy-Winged Sharpshooter Board

Red Blotch Disease under the Microscope: Symptomatology of the Infection Bhaskar Bondada,\* Marc Fuchs, Andrew Walker, and Paul Skinner \*Washington State University, 2710 Crimson Way, Richland, WA 99354 (bbondada@wsu.edu)

One key aspect of all viruses, including Red Blotch, caused by *Grapevine red blotch associated virus* (GRBaV), is their intimate association with cell components and the formation of unusual structures following infection. Therefore, the objective of this study



was to characterize grapevine responses to Red Blotch disease, which is fundamental to understanding disease progression and the mechanisms by which the virus causes the infection, its infection cycle, and how the virus survives in vine and vineyard. We employed various techniques of microscopy of healthy and infected vines to understand what processes mediate infection. Cellular analysis revealed callose (a carbohydrate substance) deposited in the phloem sieve pores of afflicted vines. Healthy vines did not accumulate callose in their phloem tubes, which could be used as a diagnostic indication of Red Blotch. Similarly, in the context of vascular blockage, the xylem vessels of afflicted canes showed tylosis. These observations indicated that even though the GRBaV enters grapevines via the phloem, both xylem vessels in radial multiples, a dense cytoplasm, particles on the periphery of phloem tissues, and numerous plasmodesmata. The healthy vessels were open, and the phloem cytoplasm was mostly confined to the periphery. Such a wide range of knowledge is undoubtedly necessary to deliver effective management solutions in the near future.

Funding Support: Oregon Wine Board

#### Stomatal Conductance as a Guide to Scheduling Irrigation in Grapevine

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Stomata act as gateways for molecules of transpiration  $(H_2O)$  and photosynthesis  $(CO_2)$ . Thus, the density, pore size, and vapor conductance of leaf stomata influence rates of these key processes. Oscillations in stomatal characteristics occur in response to changes in environmental conditions, including soil water availability. Therefore, stomata may serve as indicators of plant water status. In this study, grapevine varieties Merlot and Chardonnay were subjected to varying irrigation treatments: well-watered (WW, near 100% Et\_), moderate stress (MS, 50% WW), and severe stress (SS, <20% WW). Stomatal conductance was measured weekly from bloom until fruit maturity. The same leaves analyzed for stomatal conductance were immediately measured for midday leaf water potential. As water stress increased, both stomatal conductance and midday leaf water potential decreased. A linear relationship between the two measurements existed under all conditions, indicating that stomatal conductance responds in a similar manner to leaf water potential and could be used as an irrigation scheduling tool. The effects of water stress on stomatal density and stomatal pore aperture were also evaluated. Using imprints made with nail polish, the density of stomata/mmĐ leaf area was determined using light microscopy. Mean stomatal density varied between the two varieties, with Chardonnay being higher than Merlot. Interestingly, WW Chardonnay leaves had a significantly lower stomatal density than SS leaves of the same variety. This indicates that stomatal conductance measured by a porometer can be used to detect water status in grapevines and has potential as an irrigation scheduling tool.

Funding Support: Northwest Center for Small Fruits Research



2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

## Effects of Soil and Topography on Water Status of Chardonnay and Meta-Analysis of the $\delta^{13}C/Water$ Potentials Correlation

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The measurement of carbon isotopic discrimination in grape sugars at harvest ( $\delta^{13}$ C) provides an integrated assessment of water status during ripening. It is an efficient alternative to assess variability in the field and to discriminate between management zones in precision viticulture, but further work is needed to completely understand the signal. This work, spanning over three years and performed in eight different plots in a hillslope toposequence in Burgundy, delineates the relationships between primary soil properties (gravel amount, slope, texture) and grapevine water status assessed by  $\delta^{13}$ C and predawn leaf water potentials ( $\Psi_{\rm pd}$ ). Brix and tartaric and malic acids were also measured. The greatest  $\delta^{13}$ C, indicating the most severe water deficit, was recorded in gravelly soils on steep slopes. The amount of sugars and malic and tartaric acids was also related to  $\delta^{13}$ C. The relationship between  $\delta^{13}$ C and  $\Psi_{nd}$  was also investigated, because the absolute values of measured  $\delta^{13}$ C were lower than values currently found in the literature. A mini-meta-analysis was performed, which showed that the slope of the relationships between minimum  $\Psi_{\rm pd}$  and  $\delta^{\rm l3}{\rm C}$  was stable across studies (a change of 1‰ in  $\delta^{13}$ C corresponded to a change of D0.2 MPa in the minimum  $\Psi_{nd}$ ), while the intercept of the comparison  $\delta^{\rm \scriptscriptstyle I3}{\rm C}/\Psi_{\rm _{pd}}$  changed, probably because of genetic variations between varieties or environmental differences.

Funding Support: Bureau Interprofessionel des Vins de Bourgogne

### Deficit Irrigation and Mechanical Leafing on Yield, Berry, and Wine Composition of Cabernet Sauvignon Grown in California

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Cabernet Sauvignon is one of the most important winegrape varieties grown in California, and >25% of California's Cabernet Sauvignon was grown in the San Joaquin Valley (SJV) in 2016. The SJV is not commonly an ideal environment for growing premium Cabernet Sauvignon, but high yields of acceptable fruit are possible and necessary to stay competitive. To help achieve these goals, we established a field experiment to study the effect of regulated deficit irrigation and mechanical leafing on Cabernet Sauvignon using a two-way factorial split-plot design in a commercial vineyard located in western Madera County in 2018. Cabernet Sauvignon vines grafted onto Freedom rootstock were trained to quadrilateral cordons and spur-pruned under a sprawl system with 5 D 10 ft vine and row spacing. Two levels of water deficit and three timings of mechanical leafing, replicated five times, were used in this study. The two levels of water deficit were 80% ETc throughout the growing season and 50% ETc from berry set to veraison and 80% ETc after veraison. The three timings of mechanical leafing were bloom, berry set, and no leafing. Midday leaf water potential ( $\Psi$ ) and fruit-zone photosynthetically active radiation (PAR) were measured to assess vine water status and fruit-zone light exposure during the season. Water deficit significantly decreased vield, through reduced cluster number and berry size, and lowered pruning weight without changing leaf area. As for berry composition, water deficit reduced titratable acidity and IBMP while improving berry anthocyanins. Leafing didn't change



2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

yield components, but improved berry anthocyanins and increased IBMP when conducted at bloom. Wine color was improved by water deficit and leafing at bloom.

Funding Support: None

#### Impact of Mechanical Leafing on Yield and Berry Composition of Ruby Cabernet Grown in the San Joaquin Valley of California

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Ruby Cabernet, a cross between Cabernet Sauvignon and Carignan, can produce wines with high color and tannin under the arid, warm growing climate of the San Joaquin Valley, California. Local growers and wineries mainly use this variety to increase the color and tannin of varietal wines such as Cabernet Sauvignon and Merlot. Growers expect high yields of fruit with high levels of anthocyanins and tannins, but that is not always the outcome. Therefore, in 2018, an experiment was conducted in a commercial vineyard located in western Fresno County to determine how deficit irrigation and leafing affect yield and fruit quality. Quadrilateral cordon-trained, spur-pruned Ruby Cabernet vines grafted onto Freedom rootstock were used under a sprawl system. Four timings of mechanical leafing, replicated five times, were used in the study, with five vines designated as an experimental unit. Deficit irrigation was kept at 80% ETc from berry set to veraison and decreased to 60% ETc from veraison to harvest. Four timings of mechanical leafing included: bloom, berry set, veraison, and no leafing. Midday leaf water potential ( $\Psi$ ) and fruit-zone photosynthetically active radiation (PAR) were measured to assess the vine water status and fruit-zone light exposure during the season. Leafing at berry set and veraison reduced cluster weight through decreased berry number/ cluster, directly resulting from physical leafing damage, and leafing at bloom and berry set improved berry anthocyanins, but leafing at veraison did not. None of our leafing treatments impacted per vine yield, berry size, and pruning weight in 2018.

Funding Support: None

#### Mist-Type Evaporative Cooling System to Control Grapevine Canopy Temperature during Heat Waves

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The frequency of extreme weather conditions is increasing due to climate change. Recurring heat waves are challenging grape production because excessive heat stresses vines in vineyards where regulated deficit irrigation is being applied. Water-stressed vines cannot remove excessive heat efficiently by transpiration. To mitigate heat stress, several methods have been tried in the past. Though shade netting or overhead sprinkler irrigation mitigated excessive heat, grape quality, yield, and plant health were compromised. In 2018, we built a novel cooling system in a Cabernet Sauvignon block in southeastern Washington. This mist-type evaporative cooling system (MECS) only provides enough water to the canopy to decrease canopy temperature ( $T_c$ ) by evaporation. Misting was activated when  $T_c$  was above 35°C and leaves were dry and deactivated when  $T_c$  was below 33°C or leaves were wet. The impacts of MECS were compared to vines without MECS coverage. During a heat wave in July,  $T_c$  was contained below 37°C when the surrounding air temperature ( $T_a$ ) was 40°C and  $T_a$  in the canopy was 42°C. When the atmosphere relative humidity (RH) was 15%, RH was 16 3 1% in

### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

MECS canopy, which was greater than in the control canopy (12 3 0%). Shoot length, fruit yield, juice total soluble solids, pH, and titratable acidity showed no differences between treatments. The daily water usage was equivalent to precipitation of 0.4 mm. Neither berry splitting nor disease incidence was observed in either treatment. The MECS demonstrated its ability to decrease  $T_c$ .

Funding Support: USDA Specialty Crop Block Grant Program; Washington State Grape and Wine Research Program

# Site Delineation by Plant Water Status and Sugar $\delta^{13}\text{C}$ Analysis in a Fully Mechanized Vineyard in Napa County

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Vineyard ecophysical variations limit vineyard efficiency in production and berry chemistry. A fully mechanized Cabernet Sauvignon vineyard was modeled to investigate the ecophysical variability in Napa County, California, including soil electric conductivity (EC), plant water status, carbon isotopic discrimination analysis ( $\delta^{13}$ C), and berry primary and secondary metabolism. The time-independent  $\delta^{13}$ C analysis can be used as an alternative approach to assess plant water status. In this study, an equidistant grid sampling was performed. Stem water potential ( $\Psi_{ ext{stem}}$ ) and leaf gas exchange were measured weekly during the growing season, and their integrals were calculated.  $\delta^{13}$ C was measured at harvest and was directly related to  $\Psi_{stem}$  (R<sup>2</sup> = 0.69), stomatal conductance ( $R^2$  = 0.27), carbon net assimilation ( $R^2$  = 0.25), and water use efficiency (R<sup>2</sup> = 0.67). Soil EC only showed a relationship with  $\Psi_{\rm stem}$  at a soil depth of 0.75 m (R<sup>2</sup> = 0.23).The vineyard was separated into two zones by k-means clustering in  $\Psi_{\text{stem}}$ : Zone 1 with greater water stress and Zone 2 with less water stress. There was no difference in total soluble solids, titratable acidity, or pH. Skin and berry weight were greater in Zone 2, but yield and cluster number/vine were the same. Zone 2 had more peonidin- and malvidin-3-glucoside content/berry but a lower tri- to di-hydroxylated anthocyanin ratio. There was no difference in flavonol concentration. Our results provided evidence that delineating vineyard by plant water status, using  $\delta^{13}$ C as a reliable assessor, and assessing ecophysical variables using a precision viticulture approach without traditional labor input are viable.

## Micro-Tensiometer Sensor for Continuous Monitoring of Stem Water Potential in Grapevines

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Recently developed micro-tensiometer (MT) sensors were installed in mature Cabernet Sauvignon vines in the UC Davis vineyards starting in August, 2018. A total of 14 sensors were installed on six vines. The vines were minimally drip-irrigated, had not been irrigated for about three weeks, and received no irrigation once the sensors were installed. Essentially all sensors exhibited similar relative daily patterns in SWP that were comparable to patterns shown by the pressure chamber. However, there was large sensor-to-sensor variation in the range of water potential values reported. For these vines, the relative values of the sensors indicated that the highest SWP did not occur at predawn (prior to 0600 hr in August), as is commonly assumed, but rather about an hour after dawn. The midday minimum water potential (maximum stress)



occurred -1500 hr, about two hr after peak reference evapotranspiration (ETo). These findings are consistent with independent in situ measurements of SWP in other woody perennial species. Each MT was calibrated against the corresponding pressure chamber-measured SWP on an individual vine basis, giving consistent MT measured SWP values for five of the six vines in the study. The average values from these vines showed a very clear pattern of overnight recovery to about -3 bars from August to November, when rains caused an increase to above -2 bars. Midday SWP values were around -7 bars, also recovering with normal seasonal leaf loss and rains. Midday MT-measured SWP also fluctuated in parallel with the weather-related baseline (fully irrigated) SWP for about three months, indicating the possibility for continuous monitoring over relatively long periods. These data illustrate that automated and sensitive monitoring of SWP in grapevine is possible with these sensors and should provide useful information for irrigation management and physiological studies.

Funding Support: AVF

#### Meta-Analysis of Plant Water Relations in California Vineyards

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Grapevine is among the largest commodities in California, and because the growing season occurs during the dry spring and summer months, this crop is irrigated. With increasingly warm temperatures, the amount of water required for irrigation may also increase in the attempt to cool grapes on the vine and reduce loss in quality because of heat stress or excessive drought stress. Optimizing irrigation is not only important for environmental concerns, but also because it directly affects yield and composition of grapes and wines. This work presents the results of a meta-analysis of grapevine water relations and their effects on yield and grape composition in California vineyards. We performed a comprehensive search of the published, peer-reviewed literature reporting irrigation treatments, plant water status, and leaf gas-exchange together with effects on yield and grape composition. All selected articles described work performed in California within the last 20 years. Weather data were obtained from the closest California Irrigation and Management System when not provided directly. Soil data were obtained from the USDA soil web database when accurate geographic coordinates were provided within the study and if soil data were not included in the original work. The resulting database contained several hundred data-points from the main Vitis vinifera varieties and the major rootstocks grown in California. The main factors affecting grapevine response to decreasing water amounts were analyzed with respect to the production district, variety, soil, and rootstock. Statistical analysis was also performed to clarify the effect of weather extremes on plant water status and leaf gas exchange at the California scale. Linking environmental and agricultural parameters, a model was developed to predict plant water status and understand its critical effects on yield, to provide a unified, comprehensive vision of the effects of water stress on the grape industry in California.

Funding Support: California State University - Honor program

WED/THUR POSTER ABSTRACTS

### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

### Prediction of Phenological Stages and Cultivating Support System for Winegrapes in Japan

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Interannual variability of weather conditions causes large variations in the dates of winegrape phenological stages, which hinders the grape producer's field management. Phenological and quality data of winegrapes was collected intensively from major production areas in Japan (Yamanashi, Nagano, and Hokkaido prefectures) from 2016 to 2018. First, we tried to build an estimating equation for important phenological stages to be used in cultivation management using the developmental-rate model. The estimation accuracy for the flowering stage was 2.4 days in the RMSE over 10 varieties (e.g., Chardonnay, Pinot noir, and Koshu), while that for veraison was 5.8 days. Second, to convey the result to grape producers, an experimental information system was built. The system uses the online observation data set on grape fields and weather forecast data to estimate the flowering and veraison dates. The system will be released commercially in spring of 2019 in Japan.

Funding Support: The Project of the NARO Bio-oriented Technology Research Advancement Institution (the special scheme project on regional developing strategy)

# Sulfur: A Broad Perspective on Why It's Time to Optimize Applications in Winegrowing

#### Eve-Lyn Hinckley\*

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Human changes to sulfur (S) cycling have shifted dramatically in recent decades. Once dominated by mining and fossil fuel emissions that caused inadvertent inputs of reactive S as acid deposition to forested ecosystems, our major pathway of S manipulation is now the multiple forms used in agriculture. While many decades of research have documented the effects of atmospheric S deposition to acid-sensitive forest and aquatic ecosystems, comparatively little is known about the unintended consequences of its use in large-scale agricultural systems, including California vineyards. This presentation highlights the fates of S in crop soils and adjacent ecosystems. I include illustrations of three regional crops in the United States: winegrapes in California, where S is used as a fungicide, corn in the Ohio River Valley, where it is used as fertilizer, and sugarcane in Florida, where it is used to regulate soil pH. Long-term datasets from these areas demonstrate that agricultural S inputs have increased with expansion and intensification of crop systems. In some cases, S applications have increased in response to federal regulation of emissions, which reduced the "free" supply of atmospheric S deposition. Analysis of agricultural S in the context of a changing climate-particularly during prolonged drought in California-demonstrates the tight link between hydrology and the residence time of S, defined as whether it remains on-site and contributes to soil acidification and losses of base cations or moves off-site and stimulates bioavailability of metals in wetlands and streams. Ultimately, it is important to work toward optimizing S applications in major agricultural areas, both to meet crop needs and minimize environmental consequences. Ongoing studies in California winegrowing are evaluating



2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

how to improve powdery mildew forecasting models, detection, and S spray efficiency, positioning this industry to lead in sustainable S management.

Funding Support: The National Science Foundation

# Trends in Long-Term Winegrape Phenology: Can They Be Explained by Changes in Microclimate?

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Phenology, or the timing of life stages, is sensitive to climate change across ecosystems and species. It is a critical component of plant success, influencing plant production and interactions with other species like pests and pollinators. While climate-mediated changes in winegrape phenology have been documented in multiple regions and some varietals show associated trends toward higher potential wine quality, the controls on and consequences of phenological shifts for grapevines are understudied. Further, while certain winegrape varietals are more phenologically responsive than others, we lack a predictive framework for how winegrapes will alter their phenology in response to climate change across environmentally variable landscapes, such as across gradients of soil moisture. The degree of climate change experienced, termed "climate exposure" in ecological theory, may help us explain these past phenological phenomena and predict future patterns. We hypothesize that topographic features and vine structuring (e.g., vine row orientation, location within a block, or canopy structure) can regulate the degree of climate exposure experienced, variations of which may either prevent or exacerbate phenological shifts. Here, we pair new environmental data with a 20-yrlong phenology data set from Cain Vineyard in Napa, California, to test whether (1) microclimate differences can predict long-term trends in grapevine phenology, and (2) changes in grapevine phenology influence grape production. We report fluctuations in block-scale phenology across years and between varietals and propose potential environmental explanations for these trends. While regional patterns in climate exposure have helped predict the strength of phenological shifts, this work is a novel attempt to explain long-term trends in grapevine phenology at the spatial scale of a block. Thus, this research contributes to our ability to project phenological shifts under future climate change, improving predictions of future phenology-mediated changes in grapevine production and wine quality.

Funding Support: Environmental Biogeochemistry Group at CU-Boulder

### Impact of Foliar Inactive Dry Yeast Application to Chambourcin Grapevines on Wine Anthocyanin Content

Sarah Mayfield\* and Renee Threlfall \*University of Arkansas, 2650 N. Young Ave, Fayetteville, AR 72704 (sem013@uark.edu)

LalVigne (Lallemand, Inc.), an inactive dry yeast rehydrated and applied foliarly to grapevines, can increase anthocyanin content of *Vitis vinifera* winegrapes. The impact of foliar LalVigne application to Chambourcin grapevines on wine anthocyanin content was evaluated. Chambourcin grapevines were grown in a commercial vineyard in Arkansas. Four rows of Chambourcin were sprayed with LalVigne MATURE at veraison and 10 days later (sprayed), four rows were unsprayed (control), and six rows were between as a buffer. Grapes were handharvested August 2018 in four 50-kg lots from each spray treatment. The grapes from both treatments had commercially acceptable soluble solids

### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

(21.1%), pH (3.6), and titratable acidity (0.58%) at harvest. The eight 50-kg lots of grapes were crushed separately. Tannin (800 mg/L) and oak chips (8 kg/t<sub>m</sub>) were added to two 50-kg lots of must from each spray treatment, and two were without additions (additions and no additions, respectively). The wines were fermented on the skins for five days and pressed, then fermentation was completed at 15°C. The wines had final pH levels of 3.36 to 3.50, titratable acidity of 0.70 to 0.74%, and 10.5% (v/v) ethanol. Wines were evaluated at bottling in February 2019 for the predominant anthocyanins (malvidin-3-glucoside and petunidin-3-glucoside) and total anthocyanins. Wines from sprayed grapes had greater malvidin-3-glucoside (21.3 mg/100 mL) than wines from control grapes (20.4 mg/100 mL). For wines with additions, wine from sprayed grapes had more petunidin-3-glucoside (8.90 mg/100 mL) and total anthocyanins (108.4 mg/100 mL) than wine from control grapes (8.23 and 102.9 mg/100 mL, respectively). For wines without additions, there was no difference in wine made from sprayed or control grapes for petunidin-3-glucoside (7.2 mg/100 mL) or total anthocyanins (100.6 mg/100). Application of inactive dry yeast to Chambourcin grapevines produced wines with greater amounts of red-colored anthocyanins.

Funding Support: Lallemand, Inc., Danstar AG, Scott Laboratories

# Impact of Foliar Grapevine Application of an Inactive Dry Yeast on Chambourcin Grapes from Veraison to Harvest

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LalVigne (Lallemand, Inc.) is an inactive dry yeast that is rehydrated, and when applied to grapevines as a foliar spray, has been shown to increase skin thickness and anthocyanin content of Vitis vinifera winegrapes. Foliar grapevine application of LalVigne on Chambourcin, a French-American hybrid, was evaluated for grape berry composition, physical properties, and anthocyanin content. Chambourcin grapevines (eight to 10 years old) were grown on a single bilateral cordon trellis system (200 m rows) in a commercial vineyard in Arkansas (cold hardiness zone 6b). Four rows of Chambourcin were sprayed with LalVigne MATURE at 5% veraison and 10 days after veraison (sprayed), and four rows were left unsprayed (control), with a six-row buffer between the rows. Two hundred grapes were handharvested from the grapevines in triplicate from each treatment once/week in 2018 from veraison (20 July) to harvest (27 Aug). The grapes from both treatments ripened as expected, but there were differences between sprayed and control grapes prior to and at harvest. Grapes from both spray treatments had commercially acceptable harvest composition for wine production. At harvest, there was no difference between grapes from sprayed and control grapevines in soluble solids (21.1%) or titratable acidity (0.58% as tartaric), but control grapes had higher pH (3.64) than sprayed grapes (3.52). Grapes from sprayed grapevines had greater skin elasticity (7.07 mm) and lower berry weight (2.05 g) than control grapes (6.38 mm and 2.11 g, respectively) across all sample dates. At harvest, grapes from sprayed grapevines had more total anthocyanins (302.7 mg/100 g), malvidin-3glucoside (82.4 mg/100 g), and petunidin-3-glucoside (67.7 mg/100 g) than control berries (265.8, 67.5, and 58.5 mg/100 g, respectively). Application of LalVigne inactive dry yeast to Chambourcin grapevines produced grapes with lower pH, potentially thicker, more flexible skins, and more red-colored anthocyanin compounds.

Funding Support: Lallemand, Inc., Danstar AG, Scott Laboratories



#### Using Machine Learning to Predict Sugar Levels in Winegrapes

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We present a novel approach to predicting sugar level in grapes, based on historic sugar level progression and the current season's measurements. Predicting grape maturity, as sugar level (Brix) and other measures, is one of the most challenging decisions in grape production. While advanced methods can accurately measure sugar levels and model the chemical process of sugar accumulation, there has been little study of the best way to accurately estimate sugar levels few weeks into the future. Many growers base their decisions on simplified rules of thumb such as: "Brix rate increase is usually 1 Brix/week." We applied the paradigm of supervised machine learning over big data to this problem. We constructed a training data set in which the sugar measurements near the end of the season are used as the target variables ("labels" in the machine learning terminology). For each target measurement, we produced one training example/measurement preceding it. For each training example, we calculated attributes ("Features" in the machine learning terminology), from the training measurements. Example of useful features include the value of the training measurements, the time difference between the training measurement and the target measurement, the slope and r-square of the regression line of the training measurements, the winegrape variety, and the weather around the time of measurement. We applied a gradient tree-boosting algorithm to the above data set, obtaining a predictive model for the target measurements. Our evaluation method was a year-based cross-validation. The model was trained separately on the full data set excluding one year, and then applied to the held-out year. The RMSE (Brix units) of our predictions on the held-out data was 1.37 and its coefficient of determination (R-Square) was 0.53. This is compared to RMSE = 1.72 and R-square = 0.48 achieved by a naive prediction of 1 Brix increase/week.

Funding Support: Trellis Inc.

### New Application for the Improvement of Vineyard Growth Parameters Through the Reactivation of the Soil Microbiome

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With sustainability as an ever-increasing priority with grapegrowers, more innovative soil fertilizing materials will prove indispensable to meet the challenges of modern agriculture in a changing climate, while simultaneously minimizing the impact of these agricultural systems on the environment. BluVite was developed as a multi-purpose product for all vine varieties, for use in both mature vineyards and new plantings. Due to its particular composition and origin, BluVite helps facilitate the biological mechanisms that reactivate the unique microbiome of the vine. Moreover, the processes of selection and interaction between the vine-specific microbiome and the soil are activated, with evident effects in the emission of secondary roots and strong adaptation to the biotic and abiotic stresses of the plant. General morphological parameters evaluated included, but were not limited to, diameter and length of shoots, SPAD and leaf area index (LAI), uniformity of internodes, development of the shoot tip, resistance to water stress, average cluster weight, average berry weight, and number of seeds/berry. Laboratory analysis of the grape juice and resulting wines was also carried out to assess the effects on the grape clusters (and wines) and includes a whole suite of tests including pH, K+, TA, Brix, and anthocyanin content, to name a few. Furthermore, with the application of BluVite, we have seen improvements in plant growth and grape maturity, including more uniform and robust canopy growth and improved cluster development. In this manner, it

### Wednesday & Thursday National Conference Poster Presentation Abstracts (Research Reports)

2019 NATIONAL CONFERENCE TECHNICAL ABSTRACTS CONTINUED

### Enology and Viticulture Research Report Posters—CONTINUED

is possible to stabilize the soil ecosystem in a sustainable way, while increasing targeted plant growth parameters and the quality and quantity of grapes produced.

Funding Support: Enartis USA

# Characterization of the Oxygen Transfer Rate of "New-Ancient" Natural Materials in Wine Maturation Containers

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Today there is a trend in oenology that promotes the use of old natural materials used in the past for the manufacture of storage and maturation wine tanks. One of the most sought-after characteristics of these materials is their permeability to atmospheric oxygen, allowing them to improve wines without using harmful processes. The reference performance is the oak barrel for its ability to oxidize wines in a controlled way to improve them. In this way, it would be possible to carry out the process of maturing wines in containers in which the use of wood is not obligatory, as is the case with oak barrels or foudres. Presented in this work are the results of oxygen permeation analyses under test conditions typical of a tank of materials such as stoneware, ceramics (qvevri), concrete, and granite. The natural materials can be divided into two classes, which are formulated based on different components such as ceramics (qvevri), gress, and concrete (considered a natural material). On the other hand is stone, which, although it is a composite material, has a composition that is not modifiable and depends on its origin. The oxygen permeability of the materials tested has been very diverse, typical of natural materials. The results show that ceramic (qvevri) presents an excessive permeability, not only to atmospheric oxygen, but also to liquids and needs a treatment to be able to be used in liquid containers. Gress and concrete can be impermeable to liquids, but they maintain a permeability to atmospheric oxygen that makes them candidates for use in permeable tanks for wine aging. Finally, granite has some very interesting characteristics; however, it is necessary to use thickness control for the desired oxygen transmission rate.



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