66
SIXTYSIXTH NATIONAL CONFERENCE

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TECHNICAL ABSTRACTS

66th ASEV National Conference

June 15–18, 2015

Portland Marriott Downtown Waterfront Hotel

Portland, Oregon USA

AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

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Enology — General Enology/Fermentation Session

Pressing under Inert Gas—Impact on Chemical and Sensory Characteristics of Sauvignon blanc and Riesling

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Inert gas pressing aims to reduce the oxygen uptake in must. By limiting oxygen exposure, oxidation processes are slowed down and grape content is presumably protected from destructive effects. Conversely, it is known that aroma precursors are not easily oxidizable, and recent studies showed that oxidative conditions in must can even favor the formation of varietal thiols. Our objective was to compare chemical and sensory properties of Sauvignon blanc and Riesling musts and wines obtained from pressing under inert gas (50 hL) and traditional pneumatic pressing (50 hL). Hand-picked and machine-harvested grapes from 2012 and 2013 with different SO_2 and ascorbic acid additions were vinified under reductive or nonreductive conditions. Must oxygen concentrations after inert gas pressing were decreased by 90 to 99.9%, depending on the press loading level and loading system. While musts from traditional pressing showed browning with increasing pressure, inert gas pressing preserved the green color until the last pressure cycle. Glutathione and hydroxycinnamic acid concentrations were 20 to 40% higher after inert gas pressing. Despite the suspected stability towards oxidation, the precursors of C_6-alcohols and aldehydes and monoterpenes were also found in higher concentrations. Descriptive analysis revealed more green and fruity character and higher minerality in Sauvignon blanc and Riesling wines which were kept under reductive conditions from grape to bottle. While these changes were reflected in increasing typicality ratings for Sauvignon blanc, Riesling was judged atypical after bottling. For both Riesling and Sauvignon blanc, the omission of reductive conditions during winemaking/bottling caused unfavorable browning of wines and a decrease in varietal aroma. This was probably due to the preservation of easily oxidizable phenolics in must obtained from inert gas pressing. Sauvignon blanc wines most reminiscent of thiol aroma were obtained by traditional pneumatic pressing, ascorbic acid addition to must, and reductive winemaking/bottling.

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

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Enology — General Enology/Fermentation Session – CONTINUED

Role of Nutritional Deficiencies, Oxidative Stress, and Insufficient Sulfur Dioxide Levels in Sluggish Fermentations

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Judicious management of fermentation nutrients, microbial competition, and strain selection dramatically reduces the risk of sluggish or stuck fermentation. Sulfur dioxide is added to must for its antimicrobial and antioxidant properties. However, instances of sluggish and arrested fermentations arise periodically in spite of careful management. Our study focused on the factors in juices that negatively impact strain fermentation performance. Our analysis of problematic juices revealed that they contain a high proline to arginine (pro:arg) ratio and a low yeast assimilable nitrogen (YAN) which was not alleviated by nitrogen supplementation. In addition, metabolomic analysis showed that yeast from these fermentations accumulated increased cytoplasmic mannitol levels, an indicator of reactive oxidative stress (ROS), as compared to normal juices. Certain bacterial strains found in grape juice induce the yeast to a [*GAR*$^+$] prion state, which confers the ability to use alternate carbon sources and increased viability, but which negatively impacts fermentation performance. Results from varying the levels of SO_2 showed that lower levels of sulfur resulted in higher frequencies of [*GAR*$^+$] induction. This indicated that lower levels permitted bacterial growth, allowing them to influence the metabolism and fermentation behavior of the yeast. We also studied the combinatorial role of specific nutrient deficiencies in synthetic juices, including low amino acids, vitamin deficiency, and high proline in four wine yeast strains. Results indicate that the combination of high proline and low vitamin content is inhibitory to many of the yeast strains. Thus, this work offers an explanation for the sluggishness of juices with low YAN and high pro:arg ratios—the low nitrogen is likely accompanied by low vitamin content and the correction of nitrogen levels by ammonia addition is counterproductive and the high proline exacerbates the low vitamin inhibition of fermentation. Thus, managing the combinatorial nutritional deficiencies and microbial content of the must is important for a healthy fermentation.

Funding Support: American Vineyard Foundation

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Enology — General Enology/Fermentation Session – CONTINUED

Investigation of Pump-over Frequency and Volume on Fermentation and Phenolic Composition of Cabernet Sauvignon

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Phenolic compounds in red wine are the primary contributors to the wine's color and to the sensory characteristics of astringency and bitterness. Red wines are fermented with the skins and seeds in contact with the juice so that the phenolic compounds located in the skins and seeds can extract into the wine. As fermentation progresses, the formation of the cap on the surface of the fermentor traps heat in the fermentor and limits contact between the fermenting juice and the skins and seeds. Winemakers employ different methods to mix the fermentation in order to release trapped heat and increase juice-skin contact. One such method is pump-over, where fermenting juice is pumped from the bottom of the fermentor and sprayed over the top of the cap. Experiments performed over the last three harvests (2011–2013) showed that pump-over volume and frequency had no effect on phenolic extraction or fermentation rates. However, these experiments did not investigate the low extremes of pump-over volume and frequency. The effect of low to no volume and frequency pump-overs on Cabernet Sauvignon fermentations was investigated at the UC Davis Winery during the 2014 harvest. Pump-over volumes of one-half and one-quarter fermentor volumes were investigated, as were frequencies of two, one, and zero pump-overs per day. Fermentations were performed in triplicate using research fermentors with daily sampling. Samples were analyzed by UV-vis spectroscopy and HPLC for phenolic content. Finished wines will be analyzed six months posttreatment by HPLC for phenolic content as well as by phloroglucinolysis for tannin characterization. Preliminary results show few differences in phenolic extraction between the treatments during the first five days of fermentation. After day five, significant differences were observed between the no pump-over fermentations and the remaining treatments, with the no pump-over fermentations showing a marked decrease in extraction.

Funding Support: E&J Gallo Wineries

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Wednesday National Conference
Oral Presentation Abstracts (Research Reports)

Enology — General Enology/Fermentation Session – CONTINUED

Effect of Spontaneous and Inoculated Yeast Populations on Chemical and Sensory Profiles of Pinot noir and Chardonnay

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The Okanagan valley of British Columbia (BC), Canada, has over 130 wineries, spans 8,060 acres, and stretches only 250 km. As a result, competition among local and imported wines has caused Okanagan winemakers to seek a more thorough understanding of how they can produce the most unique and sensorially desirable products. Despite risk of contamination by spoilage organisms, many BC winemakers are choosing spontaneous fermentations under the assumption that yeast populations will be more diverse and create more complex wines than inoculated fermentations. To determine how yeast populations present in inoculated and spontaneous alcoholic fermentations (replicated in triplicate) influenced chemical and sensory attributes of Pinot noir fermented in tank and Chardonnay fermented in barrel, samples from the 2013 vintage at Quails' Gate Estate Winery were collected across four stages of fermentation. *Saccharomyces cerevisiae* strains were identified using microsatellite analysis, whereas the entire yeast communities were identified using Illumina sequencing. Subsequently, samples from all replicates postfermentation were subjected to chemical analysis of fermentation-derived compounds through GC-FID and to sensory analysis by a panel of wine experts. Unexpectedly, *Pichia* ssp. were found in relatively high abundance for both Pinot noir and Chardonnay fermentations among most stages of fermentation. However, yeast population composition and diversity only differed significantly between spontaneous and inoculated Pinot noir treatments and not between those of Chardonnay. Consequently, chemical and sensory profiles of all Pinot noir wines reflected the yeast population differences found between the two treatments. Specifically, spontaneously fermented wines were found to consist of fewer dominating sensory attributes than inoculated, allowing more attributes to contribute to the sensory profiles. These results suggest that increased yeast population diversity (both *S. cerevisiae* and non-*Saccharomyces*) among spontaneous fermentations correlate with the increased complexity of the chemical and sensory profiles of wine.

Funding Support: NSERC, BCWGC, Quails' Gate Estate Winery

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Viticulture — General Viticulture Session

Effects of Biochar and Organic Floor Fertilization Practices on Vineyard Soil Greenhouse Gas Efflux and Nitrogen Status

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Biochar is a very stable form of pyrolized biomass that can be added to soil with the intention of climate change mitigation through net carbon (C) sequestration and reduction of nitrous oxide (N_2O) emissions. Both C sequestering potential and nitrogen (N) cycling effects due to biochar amendments have not been extensively studied when applied to vineyard-agro-ecosystems and some recent reports have given conflicting results. Since April 2013, we have monitored the effects of two organic floor fertility strategies (leguminous cover crops, grape pomace composted with manure) in concert with applications of biochar (applied at 1.2% of dry bulk soil weight) and grape pomace compost (applied at 1.6% of dry bulk soil weight) as carbon storage techniques. Periods of large N_2O production were largely initiated by substantial rain events. When cover cropped and compost supplemented soils were amended with biochar, significant reductions in N_2O were observed (11.3 and 30.9 $\mu g/m^2/hr$) as compared with nonfertilized (conventional) controls (79.52 $\mu g/m^2/hr$) during individual rain periods. There was minimal difference in extractable soil mineral N as ammonium (NH_4-N) among all treatments, yet a striking increase in N as nitrate (NO_3-N) in compost amended plots with biochar than those without. Interestingly, leaf lamina collected from vines located in compost treated soils had significantly more tissue N than those vines located in conventional plots. Yields have been restored to *ca 4+* tons per acre and although yields for the nonconventional treatments were significantly higher in 2013, they did not differ from conventionally fertilized plots in 2014. We did not observe any negative effects in vine vigor or berry quality due to increased vine available N. This study suggests that organic floor management for N used in concert with biochar amendments can increase NO_3-N provisions while not affecting NH_4-N and decreasing vineyard scale N_2O emissions.

Funding Support: American Vineyard Foundation

An Update on the Performance of the GRN Rootstocks

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The GRN rootstocks were released and patented from the UC Davis grape breeding program in 2009. These rootstocks were the result of many years of testing and are resistant to *Meloidogyne incognita* Race 3 (root-knot nematode), *M. incognita* HarmC, *M. arenaria* HarmA, *Xiphinema index* (dagger nematode); these four nematodes in a combined inoculum; and at high soil

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temperatures (about 30°C) where resistance to root knot nematodes often fails. They have also been tested for resistance to ring, citrus, lesion and pin nematode, and grape phylloxera. These rootstocks are now planted across California. Data from the fourth year of a trial in Lodi, as well as other plots will be presented. The Lodi trial compares GRN-1, GRN-2, GRN-3, GRN-4, and GRN-5 to eight other rootstocks (RS3, RS9, 101-14Mgt, 3309C, Harmony, O39-16. St. George, and 1103P) grafted to Malbec and grown on a quadrilateral trellis on 1.5 x 3.3 m (vine x row) spacing. Fruit yields varied significantly and ranged from 11.2 (RS9) to 18.0 kg/vine (GRN-3) as did pruning weights, which ranged from 1.53 (RS9) to 2.48 kg/vine (GRN-2). The GRN rootstocks were also evaluated for salt resistance. Preliminary results found GRN-1 to have very strong resistance.

Funding Support: California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, American Vineyard Foundation, CDFA Improvement Advisory Board, California Table Grape Commission, and Louis P. Martini Endowed Chair for Viticulture

Comparison of Benchgraft and Training Strategies on the Development and Productivity of Chardonnay Grapevines

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Plant material and training strategies utilized are critical factors in promoting vine development and production that is appropriate to the site conditions. The objective of this study was to evaluate plant material and training strategies for their potential to achieve advanced vine development and yield. A trial was established in a Chardonnay vineyard in the Salinas Valley to compare standard 30 cm long dormant benchgrafts to 90 cm vines that were produced by using a longer rootstock cutting. The 90 cm vines were trained to bilateral cordons in the first year. Standard vines were either trained to a single trunk shoot or not trained and pruned to a spur at the end of the first year. For the 90 cm vines, crop was thinned to no crop, half crop, or full crop in the second year. All treatments were evaluated for their influence on growth and productivity during the first four years of vine establishment. Full crop reduced pruning weight in the second year but did not reduce trunk or cordon diameters on the 90 cm benchgrafts. In the third and fourth years, there were no differences in yield or growth due to second year crop thinning on the 90 cm benchgraft vines. The 30 cm benchgrafts trained to a trunk or pruned to a spur at the end of year one produced smaller vines in year two and three and lower yields with vines pruned to a spur having the lowest growth and yield. By the fourth year,

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all treatments had similar crop yield, with the 30 cm benchgraft having smaller vines. The results from this trial would suggest that both plant material and vine training methods in the first year could advance the development of the permanent framework of the vine and promote the potential for earlier vine production.

Funding Support: University of California

Managing Anthocyanin Composition of Merlot Grapevine in the Hot Climate

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An experiment was conducted in the San Joaquin Valley of California on Merlot to determine the interaction of mechanical leaf removal (control, prebloom, post-fruit set) and applied water amounts [sustained deficit irrigation (SDI) at 0.8 and regulated deficit irrigation (RDI) at 0.8 (budbreak-fruit set) – 0.5 (fruit set-veraison) – 0.8 (veraison-leaf fall)] of estimated vineyard evapotranspiration (ET_c) on productivity and berry skin anthocyanin content, composition, and its unit cost per hectare. The prebloom leaf removal treatment consistently maintained at least 20% of photosynthetically active radiation in the fruit zone in both years of the study, while post-fruit set leaf removal was inconsistent across years. The RDI treatments reduced berry mass, while the post-fruit set leaf removal treatment reduced berry skin mass. The prebloom treatment did not affect yield in either year. Exposed leaf area and leaf area to fruit ratio (m^2/kg) were reduced with leaf removal treatments. The RDI treatment consistently advanced Brix in juice. Anthocyanin concentration was improved with prebloom leaf removal in both years while irrigation treatments had no effect. Proportion of acylated and hydroxylated anthocyanins were not affected by leaf removal treatments. In both years, SDI increased di-hydroxylated anthocyanins while RDI increased tri-hydroxylated anthocyanins. Prebloom leaf removal when combined with RDI maximized total skin anthocyanins (TSA) per hectare while no leaf removal and SDI produced the least. The cost to produce one unit of TSA was reduced 35% with the combination of prebloom leaf removal and RDI treatments when compared to no leaf removal and SDI. This study provides information to red wine grape growers in warm regions on how to manage fruit to enhance anthocyanin concentration and proportion of anthocyanin hydroxylation while reducing input costs through mechanization and reduced irrigation.

Funding Support: American Vineyard Foundation

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Wednesday National Conference
Oral Presentation Abstracts (Research Reports)

Viticulture — General Viticulture Session – CONTINUED

Magnitude and Timing of Fruit-Zone Leaf Removal Changes Yield and Fruit Composition of Cabernet Sauvignon

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General fruit exposure recommendations prescribe post-fruit set removal of fruit-zone leaves and lateral shoots, aiming to retain ~1.5 leaf layers. The rationale for attendant fruit exposure in a humid environment has been driven more by disease management than by documented changes in fruit composition. It was our goal to see if unconventional timing or magnitude of leaf removal would improve wine quality potential or alter yield components in Cabernet Sauvignon. Two separate randomized complete block designs were used to evaluate prebloom removal of four (PB4) and eight (PB8) basal shoot leaves (study 1) and postbloom removal of six (PFS6) basal shoot leaves (study 2) compared to no leaf removal (PBNO or PFSNO). PB8 reduced estimated crop yield by 42% and PB4 by 31% compared to PBNO; PB8 reduced berry weight by 13%, estimated berry number per cluster by 39%, estimated cluster weight by 44%, and cluster compactness (berry number per rachis length) by 59%, compared to PBNO. Prebloom leaf removal increased total berry phenolics by 23 to 26% and anthocyanins by 17 to 20%, compared to PBNO. Post-fruit set leaf removal had no effect on crop yield components or total berry anthocyanins, but PFS6 reduced total titratable acidity by 15% and increased total berry phenolics by 13%, compared to PFSNO. We speculate that even fully-exposed berries did not accumulate sufficient time at temperatures greater than 35°C to reduce anthocyanins, partially due to the high frequency of low ambient radiation during the humid growing season. Results suggest that substantial fruit exposure can be achieved in this environment without compromising wine quality potential.

Funding Support: Virginia Wine Board -National Institute of Food and Agriculture, U.S. Department of Agriculture, under agreement no. 2010-51181-21599

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Enology — Wine Tannins Session

Limits on Red Wine Tannin Extraction and Addition Part II: Role of Pathogenesis-Related Proteins in Terroir

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Polymeric flavan-3-ols, or condensed tannins (CT), impart important organoleptic qualities, such as astringency, to red wines. The extractability of CT into wines during alcoholic fermentation varies considerably among and within cultivars due to the binding of CT to insoluble grape-derived components, but the identity of these components is still not well understood. We have identified several grape-derived pathogenesis-related proteins (PRPs), including a class IV chitinase, beta-glucanase, and thaumatin-like protein, by SDS-PAGE and NanoLC-MS/MS in finished red wines. These proteins can bind and remove added CT and also likely limit CT extraction during fermentation. Proteins from 33 wines were precipitated by ammonium sulfate and quantified by SDS-PAGE. Protein quantities were highest in wines produced from wild grapes (*Vitis riparia*, 667 ± 119 mg/L), lowest in *V. vinifera* (7.3 ± 3.8 mg/L), and intermediate in interspecific hybrid cultivars (*Vitis* spp. x *V. vinifera*, 105 ± 49 mg/L). Wine PRP concentration was also inversely correlated ($p < 0.05$) with the percentage of tannins retained following tannin addition. Because PRP expression is dependent on genetics and can also be induced by biotic and abiotic environmental stresses (disease pressure, cold), we hypothesize that it contributes to variation in tannin extraction observed across and within regions.

Funding Support: USDA SCRI Project Number 2011-51181-30635

Tannin Structure-Activity Relationships during Extraction Operations

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Tannins derived from grapes are extracted during wine production operations and are responsible for red wine astringency. Considered to be an important component of overall mouthfeel quality, previous studies have shown that the structure of extracted tannins contributes to variation in perception. Developing objective analytical tools for managing tannin structure (and by extension, mouthfeel quality) is a priority for the production industry. Historically, tannin analytical methodology has focused on concentration determination. Yet, despite the importance of tannin structure on corresponding activity, until recently, analytical methods for measuring activity have been lacking. The purpose of this study was to investigate tannin structure elements (e.g.,

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Enology — Wine Tannins Session – CONTINUED

size distribution and subunit composition) and corresponding activity using a recently developed analytical method for measuring tannin stickiness. This structure-activity relationship was investigated during a critical stage of production, maceration. To execute this study, tannins were isolated from samples of must and/or wine collected throughout maceration from five Napa Valley wineries. By varying time and maceration approach, it was considered that a reasonably large variation in commercially-relevant tannin structure would result. Tannins were isolated using low pressure chromatography and characterized by size exclusion chromatography and phloroglucinolysis. Corresponding tannin activity was determined by measuring the thermodynamics of interaction between isolated tannin and a hydrophobic surface. The results of this study indicate that tannin activity (stickiness) is related to its size and epicatechin-3-*O*-gallate extension subunit proportion. Other structural elements which are thought to be important drivers of tannin activity with wine age (e.g., conversion yield and pigment incorporation) were not as strongly related during this stage of production. Overall, the results of this study give new insight into tannin structure-activity relationships which dominate during extraction.

Sasha Hazel, Melanie Sherman, Rebecca Wolff and the wineries involved in this study are thanked for their assistance.

Funding Support: American Vineyard Foundation

Relationship between Wine Matrix Composition and Corresponding Tannin Concentration and Stickiness

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For red wine, tannins are considered to be of fundamental importance to quality, where they impart astringency. Sensorially, astringency is defined as the dry mouthfeel perception when red wine is consumed. Chemically, astringency is defined as the hydrophobic and hydrogen bonding interaction between tannins and salivary proteins that leads to precipitation. During winemaking, tannins are susceptible to interactions with polysaccharides and a variety of other matrix parameters, the results of which could lead to changes in mouthfeel perception. The purpose of this study was to investigate the relationship between wine matrix composition and the corresponding tannin concentration and composition in commercial wines, so that an improved understanding of how wine matrix influences tannin could be obtained. A high performance liquid chromatography (HPLC) method has been developed to measure potential for tannin hydrophobic interaction (stickiness) in order to improve our understanding of tannin structure-activity relationships. This

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HPLC method was used to analyze the tannin concentration and stickiness of 113 wines. In addition, wine matrix chemistry (e.g., residual sugar, ethanol, acidity) was also determined. The results of this study indicated that no correlation existed between wine tannin concentration and corresponding stickiness ($r^2 = 0.070$), however within some individual varieties (e.g., Pinot noir, Syrah, Petite Sirah and Sangiovese), an improved correlation was observed. Tannin concentration and stickiness were found to be inversely related to residual sugar concentration and positively related to pH and alcohol content. Overall, tannin concentration and stickiness were generally found to be clustered by varietal, suggesting that varietally-dependent matrix chemistry influences optimal tannin concentration and activity. As cell wall polysaccharide-tannin interactions have been observed, their role in tannin extraction and perception in wine is explored.

The authors wish to thank the participating wineries for furnishing wine for this study.

Funding Support: California State University – Agricultural Research Institute

Interactions between Polyphenols, Proteins, and Polysaccharides in Wine-like Model Systems

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Protein-polyphenol interactions play a very important role in wine stability assessment, especially in red varieties. Different polysaccharides can influence this interaction and are even used as fining agents to stabilize colloidal solutions. The most common examples are mannoproteins and carboxymethyl cellulose (CMC). In some cases, the mechanisms that are involved in these specific protection reactions are not thoroughly understood and can lead to unexpected problems like the unpredictable and often delayed haze formation after CMC addition to red wines. Small scale bench trials were conducted in model systems under different pH conditions to monitor specific reactions and protection mechanisms during the interaction of proteins, polyphenols, and polysaccharides. Bovine serum albumin (BSA) and egg white protein were chosen as model proteins, a commercial grape tannin extract was used as polyphenol source, and pectin, methylcellulose (MC), glucomannan, mannoprotein, alginate, and CMC were applied as model polysaccharides. Reactions were monitored in duplicates on a 50 mL scale by spectrophotometry at 860 nm over at least 30 days. Some of the polysaccharides interacted directly with proteins or polyphenols, like MC or CMC for example, causing a precipitation. Other polysaccharides, such as pectin, were shown to delay the reaction between proteins and other macromolecules for several days depending on their concentration, thus delaying precipitation and haze formation. The use

*indicates corresponding author

of BSA as a model protein is suggested in the literature for many applications around wine. In this study however, it showed its limitations since the molecule experiences structural changes between pH 3 and 4, which change its reaction behavior. Egg white protein conversely, being a natural mix of 12 different proteins, proved to be better suited to mimic wine in a model system. The results of these experiments provide important insight into reaction dynamics between macromolecules that are involved in physical stability of wine.

Funding Support: no external funding

Interactions of Grape Proanthocyanidins and Wine Polyphenols with Yeast Cells and Cell Walls during Wine Aging

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There is great interest in enology for the use of inactivated yeast fractions to replace aging on lees in winemaking. In red wines, their impact on wine taste and stability is mainly attributed to polyphenol adsorption by yeast cell wall mannoproteins and/or to their interactions in solution with released mannoproteins. In a previous work, we compared the adsorption of polyphenols by an enological yeast strain biomass, the biomass after inactivation and drying or autolysis followed by inactivation and drying, and cell walls recovered from mechanical disruption. Results evidenced a high capacity of whole cells to irreversibly adsorb grape and wine tannins, whereas only weak interactions were observed for cell walls. This point was quite unexpected considering the literature, and raised the question of the part played by cell walls in the yeast ability to fix tannins. In the present work, tannin location after interactions between grape or red wine tannins and yeast cells or cell walls was studied by means of transmission electron microscopy, light epifluorescence, and confocal microscopy. To determine the respective part played by cell wall components, interactions between tannins and a yeast mannoprotein fraction and a linear β-glucan were also studied in solution by means of isothermal titration calorimetry (ITC) and dynamic light scattering (DLS). Microscopy observations showed that if tannins interact with cell walls, especially cell wall mannoproteins, they mostly diffuse freely through the cell wall and plasma membrane of dead cells to interact with their cytoplasmic components. ITC and DLS confirmed the existence of interactions between mannoproteins and polyphenols, whereas no interaction could be confirmed with the model β-glucan. These results demonstrated that interactions between yeast cells and polyphenols are not limited to cell walls when dealing with dead cells, providing new information concerning tannins and yeast interactions during wine aging.

Funding Support: INRA/SUPAGRO: National Institute of Agronomic Research/ International Center for Advanced Studies in Agricultural Sciences

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Enology — Wine Tannins Session – CONTINUED

Interaction of Tannin, Acid, and Ethanol Concentration on the Temporal Perception of Taste and Mouthfeel

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Alcohol, tannin, and acid concentration are key components that impact how the taste and mouthfeel of a wine is perceived. An experiment was designed to evaluate how these components interact to alter red wine taste and mouthfeel. Washington State Merlot grapes were divided into six separate fermentors (225 L) and made into wines that had either a low or high concentration of tannins (600 or 1200 mg/L CE, n = 3). Both the low and high tannin wines ethanol (0, -0.5, and -1.0% v/v) or tartaric acid (0, 1.0, and 2.0 g/L tartaric acid), were adjusted, postfermentation, for a full factorial design. This resulted in a total of 18 treatments, split evenly between the low and high tannin concentration wines. Descriptive analysis (DA) and temporal dominance of sensation (TDS) were carried out to provide the sensory profiles of the wines. Tannin concentration showed the greatest impact on the taste and mouthfeel of the wines. Sweet, sour, bitter, astringency, drying, and astringent texture were all modified by tannin concentration, but the perception of hotness was unaffected. Acid addition altered sourness and bitterness. Under our experimental conditions, the reduction of ethanol did not significantly alter taste and mouthfeel. Acid addition and tannin concentration interacted to modify the perception of sour and hot. The TDS results showed tannin concentration dictated astringent onset and length. High tannin wines showed astringent onset approximately at expectoration and lasting throughout. The low tannin wines became astringent much later, and in the case of the low tannin, no acid, no ethanol reduction treatment, the astringent feeling was delayed for almost 90 seconds after expectoration.

Funding Support: Klipsun Vineyard

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Viticulture — Pests and Diseases Session

Identification, Incidence, and Distribution of Grapevine Viruses in British Columbia

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British Columbia (BC), with over 10,000 acres of grapevine (*Vitis vinifera* L.), is the second largest grapegrowing region in Canada, with 95% of the total production concentrated in the Okanagan Valley. Viruses and other graft-transmissible agents are major limiting factors for grapevine production worldwide. Among them, grapevine leafroll disease, associated with grapevine leafroll-associated viruses (GLRaVs), is considered to be the most economically important virus disease of winegrapes, reducing plant vigor and longevity and leading to significant yield losses and poor fruit quality. To understand the current status of grapevine viruses in BC, large scale field surveys were conducted during the 2013 and 2014 growing seasons to record the incidence of GLRaVs, *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV), *Arabis mosaic virus* (ArMV), and the recently discovered *Grapevine red blotch-associated virus* (GRBaV). A total of 1,957 random-composite and 63 target-individual grapevine samples from ten red and 12 white varieties were collected from 113 vineyard blocks in different grapegrowing regions of BC and tested for the presence of GLRaVs by DAS-ELISA. Among GLRaVs, the most common was GLRaV-3 (17.2%), followed by GLRaV-2 (5.5%), GLRaV 4-9 (4.2%), and GLRaV-1 (1.4%). GFLV incidence was detected at 0.5% from 998 composite samples, whereas GFkV was detected at 29.2% from 788 composite samples. No positives were detected for GRBaV from 119 composite and 70 targeted samples tested using PCR. Similarly, no positives were detected for ArMV from 998 composite samples. Molecular characterization and insect vector transmission studies using local virus isolates are in progress. This study aims to provide directional information to develop effective management strategies for the major grapevine viruses in BC.

Funding Support: British Columbia Wine Grape Council, with matching funds from Agriculture and Agri-Food Canada's AgriInnovation Program (AIP) – Industry-led Research and Development Stream. Additional funding provided by Agriculture and Agri-Food Canada's A-base initiative

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Viticulture — Pests and Diseases Session – CONTINUED

Interpreting a Multi-Virus Survey and Designing and Delivering Virus Sampling Protocol for Industry-Wide Benefits

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Grape virus diseases cause economic loss to industry through impacts on yield and grape quality. Vector-transmitted viruses like *Grapevine leafroll-associated virus 3* (GLRaV-3), which spreads readily, can make the use of certified material seem futile when new vineyard blocks are planted near infected blocks. We began concurrent epidemiological and sociological studies in 2011 to understand the spatial aspects of GLRaV-3 and growers' disease management. Partly in response to this work, grower workgroups have formed to manage GLRaV-3 cooperatively, but concerns over GLRaV-3 have recently been superseded by the discovery of *Grapevine redblotch-associated virus* (GRBaV) and the ensuing uncertainty over its distribution and prevalence in existing vineyards. We are currently devising sampling protocols for both GLRaV-3 and GRBaV based on the spatial characteristics of existing disease maps. Additionally, in 2014 we surveyed 112 vineyard blocks to better understand the current status of grapevine viruses in production fields in the north coast region of California. Age classes were selected to capture historical "planting booms." Age classes were: 1880-1980 (old material); 1981-1995, chosen to assess the material which replaced AXR#1 rootstock removed from production due to phylloxera; 1996-2010, representing blocks often culled in response to virus-infected field selections grafted onto rootstocks not tolerant of viruses present in the material; and 2011-2014, representing a group of "young" material, encapsulating the post-2008 recession planting boom and the GRBaV crisis. Twenty-seven to 29 blocks were randomly selected in each of the four age classes. All blocks were sampled using a simple random sampling strategy, following a "W" pattern for subsample distribution. Five to 15 subsamples were collected per block depending on acreage, and tested using qRT-PCR for multiple viruses of concern. Results are interpreted in relation to changes in virus distributions over time and the positive impact of grape certification and clean plant programs.

Funding Support: American Vineyard Foundation, California Grape Rootstock Improvement Commission, California Grape Rootstock Research Foundation, The Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board

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Viticulture — Pests and Diseases Session – CONTINUED

Elucidating the Spread of Grapevine Leafroll Disease in Newly Planted Vineyards

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Grapevine leafroll (GLD) is the most economically important viral disease in Washington vineyards. Previous studies indicate that *Grapevine leafroll-associated virus 3* (GLRaV-3) is predominant among the GLRaVs documented in GLD-affected vines. The objective of this study was to gain improved understanding of the spread of GLRaV-3 in time and space in young vineyard blocks in order to implement effective control strategies. For this purpose, we selected Syrah and Cabernet Sauvignon blocks, planted with virus-tested cuttings in 2004 and 2007, respectively, adjacent to GLD-infected old blocks. During each season, the position of individual vines showing GLD symptoms in the two blocks were recorded in a matrix representing the planting lattice. Representative samples from symptomatic vines and symptomless vines on either side of symptomatic vines were tested each season by RT-PCR for GLRaV-3 to determine the viral status of symptomatic and non-symptomatic vines. The results from multiple seasons showed a gradual increase in the number of newly infected vines over successive seasons in Cabernet Sauvignon and Syrah blocks. Spatial and temporal mapping of symptomatic vines in both blocks using ArcGIS showed a disease gradient with the highest percentage of symptomatic vines located in rows proximal to infected old blocks and the disease incidence declining with increasing distance from infected blocks. Spatial autocorrelation and hot spot analysis suggested random distribution of symptomatic vines in young blocks during initial years, indicating primary spread of the virus and aggregation/clustering within and across rows during subsequent years, indicating secondary spread between vines. Molecular analysis of GLRaV-3 sequences indicated the presence of isolates belonging to the variant group I in both Syrah and Cabernet Sauvignon blocks. The results of this study provided valuable information for implementing postplanting management strategies to control the spread of GLD.

Funding Support: Members of the Wine Advisory Committee of the Washington Wine Commission, Agricultural Research Center, WSU-CAHNRS, Altria - Chateau Ste. Michelle Wine Estates

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Viticulture — Pests and Diseases Session – CONTINUED

Aspects of Entomopathogenic Nematodes for Controlling the Vine Mealybug, *Planococcus ficus*, in South African Vineyards

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The aim of the study was to determine the potential for entomopathogenic nematodes of the families *Heterorhabditidae* and *Steinernematidae* to control *Planococcus ficus* (Signoret), the vine mealybug, within an integrated pest management scheme. The vine mealybug, which occurs on both the aerial parts and on the roots of the plant, is the dominant mealybug species and regarded a severe insect pest in the South African grape industry. The chemical control of mealybugs is restricted due to their water repellent, waxy secretions and their ability to rapidly develop resistance. Chemical exposure is limited due to the mealybugs' cryptic lifestyle of hiding within crevices, under bark, and below ground on roots. Eight nematode species were screened for their efficacy in killing adult female *P. ficus*. Highest mortalities were due to the indigenous species *Heterorhabditis zealandica* and *Steinernema yirgalemense*, with 95 and 65% mortalities, respectively. In laboratory conditions, both *H. zealandica* and *S. yirgalemense* were able to move 15 cm down sand columns to infect *P. ficus*, with mortalities of 82 and 95%, respectively. Laboratory persistence of *S. yirgalemense* in sterile, moist sand remained high (>85%), while that of *H. zealandica* dropped to 5% after six months. *S. yirgalemense* was applied to the soil of two different vineyards, with adult female *P. ficus* contained in pierced Eppendorf tubes, buried at a depth of 15 cm in the soil; mortalities of up to 50% were obtained within 48 hr. In field persistence, 12 wk after application of *S. yirgalemens,* measured using codling moth larval mortality, *P. ficus* was found to be zero in one vineyard, while 70% in the other. These studies indicate that entomopathogenic nematodes, specifically *S. yirgalemense*, have promising potential as a biological control agent for *P. ficus* soil populations.

Funding Support: Winetech (Wine Industry Network of Expertise and Technology), T.H.R.I.P. (Technology and Human Resources for Industry Program)

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Viticulture — Pests and Diseases Session – CONTINUED

Improving Our Understanding of Sour Rot Etiology and Management Techniques

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Sour rot, a disease affecting grapes near harvest, has been observed in the field for years with only limited research into its biology or control. The disease is characterized by the discoloration and rotting of grapes on the vine accompanied by the smell of acetic acid. Sour rot is routinely associated with the presence of *Drosophila* (fruit fly) species. We have successfully reproduced the field symptoms of sour rot in the lab by wounding a healthy berry, inoculating it with *Saccharomyces cerivisiae* and *Acetobacter aceti* (for production of ethanol and its subsequent oxidation to acetic acid, respectively), and simultaneously exposing the berry to *Drosophila melanogaster*, without which typical symptoms do not develop. To examine a hypothetical causal role in disease development that these insects might play, we produced axenic flies and repeated the experiment, observing that non-axenic flies were less successful in producing symptoms than comparison flies without resident microbiota, suggesting that the insects' resident microbiota are not an etiological component of sour rot. Trials in a commercial vineyard of the *Vitis* interspecific hybrid Vignoles revealed a significant effect of training system on sour rot development. In each of two different seasons, vines trained to a top wire system had an average of 22% sour rot severity versus 11 and 16% severity for those same years in the VSP system. In one year, sour rot incidence was 35% in the top wire system versus 17% in the VSP system. Spray trials integrating antimicrobials and insecticides are ongoing, to confirm our previous observation that controlling resident microflora and insects provides a synergistic effect. We also are using Illumina sequencing to examine the seasonal dynamics of yeast and bacterial populations on both grape surfaces and *Drosophila* species found at vineyard sampling sites, to aid in the understanding of disease progression.

Funding Support: New York Wine & Grape Foundation

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Viticulture — Pests and Diseases Session – CONTINUED

Combating *Erysiphe necator* in *Vitis vinifera* Chardonnay: Effectively Using Chemical and Cultural Tactics

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Biopesticides are a staple in organic management for grape powdery mildew (*Erysiphe necator*), but they can play a role in conventional programs to aid in fungicide rotation. Due to their modes of action, biopesticides are generally effective when coupled with cultural strategies. To evaluate the best cultural and chemical (rotations) technique combination, different management programs were evaluated at a 3-year-old *Vitis vinifera* Chardonnay block in 2013, and at a 20-year-old block in 2014, both at WSU-IAREC, Prosser, WA. Spray programs using plant and bacterial-based active ingredients, as well as programs that combined biopesticides in rotation with synthetic fungicides, were evaluated. Fungicide applications were made with a boom sprayer traveling at 2-3 mph. Early fruit-zone leaf removal was nested within the spray programs. Fruit-zone leaf removal was done by hand at prebloom, bloom, and four weeks postbloom. Irrespective of timing, leaf removal resulted in lower powdery mildew incidence and severity compared to treatments with no leaf removal, and significantly improved spray penetration ($F = 0.0001 – 0.02$). In some cases, spray programs consisting of only biopesticides had a significantly higher incidence and severity of disease than the unsprayed controls ($F = 0.0001$). Final harvest soluble solids and titratable acidity were significantly altered by the choice of biopesticide-based programs ($F = 0.0001 – 0.009$ and $0.006 – 0.009$, respectively); there was a direct correlation between higher disease levels and lower soluble solids/higher titratable acidity. In high-pressure scenarios, a poorly-designed spray program resulted in the same disease severity than doing nothing at all. The best performing biopesticide-based spray programs evaluated here combined the strategic use of a synthetic product during the bloom period and used biopesticides to aid in FRAC rotation during the lower disease-risk periods for fruit.

Funding Support: IR-4 Program and Washington State Grape and Wine Research Program

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Enology — General Enology Session I

Closure Consistency Continued: Chemical Analysis and Sensory Evaluation

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Today a winemaker can choose among a large variety of wine closures that have different performance characteristics. A key factor that influences the wine post-bottling is the amount of oxygen that passes through the closure over time: the oxygen ingress rate. While the average rate is the most commonly described value, the variation between "identical" closures is also quite important, especially if the variation is large enough to produce two bottles of different tasting wine. In order to address this question, a high quality Sauvignon blanc wine was bottled under 200 natural corks, 200 synthetic corks, and 200 screw caps.

In this final report, we present wine absorbance data at 420 nm that was measured in each bottle quarterly for about 30 months. The browning of the wine correlated well with chemical oxidation as measured by free and total sulfur dioxide. The data also showed steady increases in absorbance for the majority of the bottles. Natural corks had the lowest average ingress rate, but also the greatest variability. However, that variability was lower, in some cases, much lower than other reports. Synthetic corks and screw caps had lower and very similar variability. At the final stage of the study, bottles were selected for each closure from high, medium, and low oxygen ingress groups to perform a descriptive sensory analysis in order to evaluate the impact of closure variability on the taste of the wine. The chemical and sensory results will be presented.

Funding Support: Amcor, Cork Quality Council, Nomacorc and Plumpjack Group

Profiling the Nonvolatile Composition of Cognacs, Armagnacs, and American Brandies Using UHPLC-QTOF/MS

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While the aroma and flavor of newly distilled brandies is largely due to the volatile composition of the spirit, most commercial brandies are aged in wooden casks for some period of time. During aging, nonvolatile components are extracted from the casks, resulting in changes in the color, flavor, and mouthfeel of the spirit. This study evaluated the nonvolatile profiles of 19 Cognacs, 9 Armagnacs, and 6 American brandies using ultra high pressure liquid chromatography (UHPLC) coupled with quadrupole time-of-flight (QTOF) mass spectrometry (MS). The nonvolatile composition of the brandies was used to model differences among the samples using principal component and discrimi-

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nant analyses (PCA). American brandies were readily distinguished from the Cognacs and Armagnacs. Additionally, the Cognacs and Armagnacs could be differentiated from each other. The Cognacs could also be differentiated by their quality classification and brandies of the VS, VSOP, and XO classes were well separated by PCA. The compounds important for differentiating among these brandies included wood-derived phenolic compounds, wood-derived triterpenoid saponins and their glycosides, and several C_6 and larger oxidized lipids. A number of additional compounds differentiated the brandies but have not yet been identified using MS and MS/MS data alone.

Funding Support: Food Safety and Measurement Facility, UC Davis

Standard Approaches for Measurement of Free SO_2 in Red Wine Severely Overestimate its Antimicrobial Activity

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Standard approaches to free SO_2 measurement, such as aeration-oxidation (A-O), iodometric titrations (Ripper method), and flow injection analysis (FIA), overestimate the free SO_2 of red wine by several-fold due to dissociation of weak anthocyanin-bisulfite complexes during analysis. The contribution of these anthocyanin-bisulfite complexes to antimicrobial activity has been unclear. In this study, sterile filtered 4 L subsamples of white and "red" wines were prepared with increasing levels of added sulfite; the "red" wine was made from the white wine with addition of an anthocyanin-rich grape extract. Molecular SO_2 was measured by both a headspace-gas detection tube method (HS-GDT) that does not perturb anthocyanin-bisulfite equilibria ("true" molecular SO_2) and through standard A-O and FIA approaches in combination with pH ("apparent" molecular SO_2). SO_2 concentrations in the white wines were well correlated between methods, but the red wines had higher "apparent" SO_2 levels relative to "true" values, attributed to anthocyanin-bisulfite dissociation. Wines were inoculated with *Saccharomyces cerevisiae* strain EC1118 (Lallemand), and viability was determined by plating on YM media and by flow cytometry using live/dead staining for 11 points over 10 days. No significant loss in total or free SO_2 occurred over the first 32 hours for any treatment level; loss at 10 days was identical to the uninoculated controls and was attributed to chemical oxidation. Loss of viability relative to the unsulfured controls demonstrated no relationship with "apparent" SO_2 (A-O, FIA) but was strongly correlated with "true" molecular SO_2 (HS-GDT). These results suggest that "true" molecular SO_2 is a good indicator of antimicrobial status, but that standard approaches to SO_2 measurement are poorly suited for predicting the microbial stability of red wines.

Funding Support: Peter and Tacie Saltonstall Endowment and New York Wine & Grape Foundation

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Enology — General Enology Session I – CONTINUED

The Direct Effect of β-Damascenone and β-Ionone on Sensory Perception of Pinot noir Wine Quality

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Volatile compounds are responsible for driving the aroma of wine. β-ionone and β-damascenone are volatile aroma compounds associated with ripening and sunlight exposure. Because of their low perception thresholds, they may play an important role in aroma. Research has shown that β-damascenone acts as an aroma enhancing compound that elevates other aromas, while the direct impact to aroma is unclear. β-ionone has been linked with floral aromas in red wines; however, there is a specific anosmia associated with β-ionone concentration which may impact overall perception of this compound in wine. Our study examined the direct impact of β-ionone and β-damascenone on the sensory perception of Pinot noir at concentrations found in Pinot noir wine, specifically to determine if this is a suitable marker for Pinot noir wine quality. Panelists were first screened for their sensitivity to β-ionone, and those that did not have a specific anosmia were included in the wine sensory analysis. Triangle tests were used to determine if individuals could distinguish wines with varying concentrations of β-ionone and β-damascenone in a Pinot noir wine matrix. Results show that β-damascenone appears to act as an aroma enhancer compound, as with other red wines. Individuals could distinguish wines with low concentrations of this compound but had difficulties with wines with higher concentrations of β-damascenone. β-ionone acts as a significant contributor to Pinot noir wine aroma, as individuals could differentiate both the low and high concentration wines from the control. When combined, these compounds appear to have the strongest impact on Pinot noir wine aroma, specifically altering the floral and fruity aspects of the wine.

Funding Support: Oregon Wine Research Institute

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Enology — General Enology Session I – CONTINUED

Impact of Red Blotch Disease on Grape and Wine Composition and Quality

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Grapevine red blotch-associated virus (GRBaV) is the second DNA virus to be identified in grapevines, having been found throughout the white and red wine grapegrowing regions of several states since discovery in 2011. Currently, little is known as to how the virus affects both grape and wine composition and quality. While reduced sugar accumulation and altered color development in the berries of infected vines have been reported, only anecdotal evidence of the effects of the virus on the flavor and tannin structure of wines exists. During the 2014 harvest, fruit was collected from vineyards in Napa and Sonoma counties from Chardonnay, Merlot, and Cabernet Sauvignon vines that were identified as being GRBaV positive and negative. Standard grape chemistries were determined at time of harvest, as was phenolic content by the Adams-Harbertson assay. Replicated fermentations were performed for all red blotch infected and red blotch-free fruit using 120 L research-scale fermentors. Preliminary data on the grapes at harvest show that the red blotch-infected fruit had reduced sugar concentrations across the varietals with slightly increased TA values in the infected fruit. In most varietals, a decrease in total phenols, tannins, and anthocyanins (for red wine varietals) were observed in the red blotch-infected grapes. The impact of the red blotch virus on the sensory characteristics of the wines will be determined by descriptive analysis using a trained panel of judges, with wine aroma and taste being evaluated. The phenolic content of the wines at the time of sensory analysis will be determined by reverse-phase HPLC and the Adams-Harbertson assay.

Funding Support: American Vineyard Foundation

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Viticulture — Ripening Session

Decrease in Fruit Firmness Can Be Used to Predict Veraison Date in Grapevine

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The onset of ripening, also called *veraison*, involves several major processes, including softening; the resumption of growth; and a decrease in acids, sugar accumulation, and, in red varieties, pigment accumulation. Predicting veraison would be of value in viticulture, e.g., for screening genotypes in studies related to grapevine phenology or for planning vineyard operations. The onset of ripening is generally recognized to take place over a very short period of time and the conventional understanding is that berry softening is included among the processes that occur during this short period. However, recent work suggests that the decrease in fruit firmness takes place over a longer period and that softening begins before other ripening events. We hypothesized that the decrease in firmness might be used as an indicator of berry developmental stage before veraison and as a predictor of the veraison date. A new tool named the Grape Grabber was developed at UC Davis to measure fruit firmness (as elasticity) in a non-destructive manner in the vineyard. This device was used in Zinfandel and Merlot vineyards to measure berry elasticity from the early stages of fruit development until ripening. A progressive and steep decrease in fruit elasticity was observed during the 10 to 14 days that preceded the onset of ripening. Significant accumulation of sugar and pigments in the berry was observed only after a loss of about 70% of berry elasticity. The decrease in elasticity was consistent between varieties and proved to be a good indicator of berry developmental stage before veraison. The Grape Grabber can be used as a tool to predict veraison date in vineyards and to identify the physiological age of berries entering ripening. The precise characterization of specific events prior to and at veraison will support future research aimed at revealing the mechanisms that regulate these events.

Funding Support: Genome British Columbia

Timing of Ripening Initiation in Grape Berries and Its Relationship to Seed Content and Pericarp Auxin Levels

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Berries in a grape cluster (*Vitis vinifera* L.) enter the ripening phase at different times, leading to an asynchronous cluster in terms of ripening. The factors causing this variable ripening initiation among berries are not known. Because the influence via hormonal communication of the seed on fruit set and

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growth is well known across fruit species, differences in berry seed content and resultant quantitative or qualitative differences in the hormone signals to the pericarp likely influence the relative timing of ripening initiation among berries of the cluster. At the time of the initiation of cluster ripening (veraison), underripe green berries have higher seed content compared to riper berries, and there is a negative correlation between the seed weight-to-berry weight ratio (SB) and the sugar level in berries of a cluster. Auxin levels in seeds relative to the pericarp tissues are two to 12 times higher at preripening stages. The pericarp of berries with high SB had higher auxin and lower abscisic acid (ABA) levels compared to those with low SB from two weeks before veraison. In the preveraison cluster, the expression of auxin-response factor genes was significantly higher in the pericarp of high-SB berries and remained higher until veraison compared to low-SB berries. The expression level of auxin-biosynthetic genes in the pericarp was the same between both berry groups, based upon similar expression activity of *YUC* genes that are rate-limiting factors in auxin biosynthesis. On the other hand, in low-SB berries, the expression of ABA-biosynthetic and ABA-inducible *NCED* and *MYB* genes was higher even two weeks before veraison. This results in higher auxin-signaling activity that lasts longer in the pericarp of high-SB berries. In contrast, in low-SB berries, concomitant with an earlier decrease of auxin level, the features of ripening initiation, such as increases in ABA and sugar accumulation, begin earlier.

Funding Support: Oregon State University

Predicting the Fruit Quality of Cabernet Sauvignon Grapes in the Outer Coastal Plain AVA from Climate Data

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Modeling of weather effects on fruit quality of Cabernet Sauvignon, an important benchmark grape variety, was undertaken to develop an understanding of which climate factors may be associated with fruit quality in this region. Consensus ratings of grape vintage quality were compiled for 36 years from two vineyards. Fruit quality was rated as premium in 16 years, adequate in 12 years, poor in five years, and super-premium in three years. Historical weather records were used as the data for predicting the effects of temperature and precipitation on grape quality ratings. The vintage quality ratings from expert growers were analyzed by fitting a cumulative logits multiple regression model. The two most important regressors were seasonal growing degree days (base 50F) and precipitation during August. Grape quality ratings increased with increasing growing degree days and decreasing rainfall during August. These two regressors yielded a regression model of which the predicted probabilities of quality were 89% concordant with the observed ratings. The model predicts that in a year with average rainfall and temperatures for these locations, there

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Viticulture — Ripening Session – CONTINUED

would be a 52% chance of producing a premium or better vintage and a 95% chance of producing an adequate or better vintage. This information is valuable to growers in deciding whether to plant Cabernet Sauvignon and how to adjust their management practices, to winemakers in determining the best usage of grapes, and to buyers and sellers in setting prices.

Funding Support: Rutgers/New Jersey Agricultural Experiment Station

Can Accumulated Growing Degree Days Be Used to Predict Harvest Timing for Hybrid Grape Cultivars in the Midwest?

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Prediction of harvest timing is of great economic value to both producers and wineries. Scheduling harvest labor, equipment, and tank space are important considerations that can benefit from knowing when specific harvest will likely occur. Continental climate conditions are exhibited by Midwest vineyards and are typified by extremes of climate variability that complicate prediction of harvest timing projections. By examining growing degree day (GDD) records and comparing with dates of harvest in Nebraska at specified harvest parameters, we have developed a method of predicting likely harvest dates for specific hybrid cultivars. However, these dates vary with projected use of the grapes and winemakers' preferences for harvest parameter levels. For example, Frontenac, to be used for a light table wine, will be harvested at lower Brix levels than when it is to be used for a late-harvest or fortified port-style wine. Another example is Edelweiss, a white grape that is harvested at 15 Brix to avoid loss of the spritely acidity associated with high quality Edelweiss wines. We have summarized more than 15 years of harvest dates for specific geophysical/climatic zones in Nebraska and provide this information to current and new grapegrowers and winery personnel to assist them in scheduling harvest of the most popular hybrid grape cultivars.

Funding Support: University of Nebraska Agricultural Research Division; State of Nebraska Grape and Wine Board; Northern Grapes Project

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Viticulture — Ripening Session – CONTINUED

Apical Shoot Tips Mediate Reduced Root-to-Shoot Translocation of Chloride in Grapevine

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Soil salinization is an increasing concern for viticultural soils and can be mitigated through rootstock breeding. However, the genetic complexity of salt tolerance, coupled with variability due to environmental factors, has slowed this effort. To better understand cultural effects on chloride translocation to the shoot and how these factors impact screening for salt exclusion, a series of whole plant manipulations were performed. In an initial experiment, severe shoot pruning of vines grown for 88 days in a container and subsequently exposed to 50 mM NaCl for approximately three weeks was shown to lower chloride concentration 2.5-fold on average in *Vitis vinifera* cv. Thompson Seedless and in four rootstock genotypes (Riparia Gloire, 110R, 1103P, and 140Ru). To dissect how specific components of pruning can lead to reduced chloride translocation, Thompson Seedless was subjected to targeted shoot tip removal in the upper, lower, or entire canopy, independently or in combination with upper or lower canopy defoliation. Upper and lower canopy defoliation was also performed without any shoot tip removal. Following 14 days of salinization at 25 mM NaCl, all treatments that included upper canopy shoot tip removal resulted in a reduction of chloride concentration in the leaves ($p < 0.011$). Shoot tip removal from the upper canopy reduced the mean chloride concentration in the leaves by 20% compared to lower canopy shoot tip removal, but upper and lower canopy defoliations with unmanipulated shoot tips were indistinguishable. The combined results of these experiments underscore the importance of cultural uniformity in salinity screening and the confounding that may occur if experimental plants are pruned in a non-uniform manner prior to the onset of salinization with the intent of homogenizing shoot size. A regulatory role is implicated for upper canopy shoot tips in the root-to-shoot translocation of chloride.

Funding Support: E&J Gallo Winery, California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, American Vineyard Foundation, CDFA Improvement Advisory Board, California Table Grape Commission, and Louis P. Martini Endowed Chair for Viticulture

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Enology — General Enology Session II

Impact of Winery Wastewater Irrigation on Soil, Grape Nutrition, and Grape and Wine Quality

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Many wineries are interested in wastewater recovery and reuse. The use of "green" chemistries as alternatives to traditional cleaning and sanitation agents will lower the biological oxygen demand (BOD) requirements for treating the wastewater and decrease the environmental impact of winery wastewater. The first part of this project investigated the effects on grape and wine composition and quality when winery wastewater (WWW) is recycled for irrigation. Water samples were collected from both the control (irrigation) and treatment (WWW) water supplies, and soil samples were collected at varying depths from five locations within the control and treatment blocks of a Napa Valley winery. Leaf and berry samples were collected at veraison (mid-season) and at harvest with harvested grapes used for winemaking and subsequent sensory analysis. All samples were analyzed for Na, Mg, K, and Ca metals by inductively coupled plasma mass spectrometry, and the grape and wine samples were also analyzed for total phenolics and tannins. The presence of grape compounds and cleaning chemicals caused the WWW concentrations of Na and K to be six times and 55 times higher, respectively, than the control irrigation water. The soil samples showed a significant difference in only K between the treatments, while the leaf samples showed significant differences in Na, Mg, K, and Ca between the treatments but the majority of these were slight. The grape samples showed significant differences in Na and Ca concentrations while the wine showed minor but significant differences in all metal concentrations. The sensory study showed no significant difference between the finished wines.

Funding Support: Agricultural & Natural Resource Competitive Grants, Gail & Ruth Oliver Fellowship, C.O. Foerster Jr. Scholarship, Louis R. Gomberg Scholarship, Horace O. Lanza Scholarship, and Wine Spectator Scholarship

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Enology — General Enology Session II – CONTINUED

Profiling Microbial Populations in Stuck Fermentations from the 2014 Vintage

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The presence of bacteria in wine fermentations is often associated with stuck or sluggish fermentations. It is not possible to determine if the bacteria found in the wine are opportunistic and present due to the inability of the yeast to effectively out-compete them or alternatively, if the bacteria arose first and have a negative effect on the yeast that slows down or stalls the fermentation. Anecdotal evidence suggests that once the bacteria become established they can have an inhibitory effect on the yeast that makes re-starting the fermentation difficult.

The 2014 vintage in California reportedly saw an increase in stuck and sluggish fermentations. A request to members of the industry yielded more than 120 fermentation samples that were considered stuck or sluggish by the winemakers. These fermentations were delivered to the UC Davis Department of Viticulture and Enology between November 2014 and February 2015. Samples were examined by microscope for the presence of bacteria and plated for bacteria and yeast. The goal was to isolate the bacteria present and determine if the bacteria could inhibit yeast growth or induce the [*GAR*$^+$] phenotype in the yeast. Many bacteria have been shown to induce the [*GAR*$^+$] prion that has been associated with sluggish fermentations. About 75% of the samples had evidence of bacterial contamination. Only 10% had acetic acid bacteria alone, and approximately 25% had lactic acid bacteria alone, both of which were visible by microscopic examination. The remaining 40% showed mixed putative acetic acid and lactic acid bacteria. However, most of the wines showed only *Oenococcus oeni* or *Bacillus* by plating, with only about 10% of the samples yielding live presumptive acetic acid or non-*Oenococcus* lactic acid bacteria by plating. *Bacillus megaterium*, a known [*GAR*$^+$] inducer, was the most common *Bacillus* species seen.

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

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Enology — General Enology Session II – CONTINUED

Strain Diversity of Yeasts in Wine Production—Use of FTIR and PCR-based Techniques for Strain Typing

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FTIR spectroscopy is a well-known method to identify microorganisms. However, this technique is not only a tool for the identification at species level; it could be also an opportunity to discriminate yeasts on a strain level. The method is based on the irradiation of yeast cells by infrared light of different wavelengths. Different compounds of the cell absorb the infrared light and give specific absorption profiles. It is an interesting tool for typing on strain level because it considers the physiological state of a living yeast cell at a very defined stage of growing under standardized conditions. The generated spectra are used to do hierarchical cluster analysis (HCA). Dendrograms were calculated by the Average Linkage Algorithm. The classification into subclusters was done by defining a spectral distance of 0.3 as a value for separation on strain level. Strain typing by this method was tested for different *Saccharomycetes* and non-*Saccharomycetes* like *Hanseniaspora uvarum* and *Candida zemplinina*. Samples of different origin were identified on the species level first, followed by generating HCAs to discriminate on a strain level. The results of strain typing by FTIR spectroscopy and hierarchical cluster analysis are compared to PCR-based methods of strain typing, such as PCR to detect d-elements in *Saccharomycetes* and a RAPD-PCR using OPA Primers to discriminate *H. uvarum* and *Candida zemplinina* strain s. The influence of different *Saccharomycetes* and cross contaminations by a commercial *Saccharomyces cerevisiae* starter culture could be shown. Additionally, the distribution of different strains of non-*Saccharomyces* species in the winemaking process could be demonstrated. In conclusion, FTIR spectroscopy is a suitable technique for discrimination of yeasts on a strain level.

Funding Support: AiF/FEI

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Viticulture — Water Relations Session

Effect of Water Deficits on Berry Growth and Composition of Seventeen Winegrape Cultivars in the San Joaquin Valley

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With increasing competition for water resources, more detailed information regarding cultivar-specific physiological responses to drought is needed for informed irrigation management decisions. An existing winegrape variety trial at the Kearney Agricultural Research and Extension Center in the San Joaquin Valley was used to investigate the berry growth and composition responses through the imposition of water deficits. Seventeen red winegrape cultivars were subjected to two regulated deficit irrigation treatments, as well as one sustained deficit irrigation treatment as a control. All treatments were irrigated at 50% of estimated crop evapotranspiration (ET_c) until berry set. An early deficit treatment received no applied water from berry set until veraison, and then was irrigated at 50% of ET_c from veraison until harvest. A late deficit treatment was irrigated at 100% ET_c from berry set until veraison, and then received no applied water until harvest. The sustained deficit treatment was irrigated at 50% of ET_c from berry set until harvest. Irrigation treatments significantly affected pre- and postveraison vine water status, canopy development, berry growth, sugar accumulation rate, and phenolic composition. In general, when considering all years and all cultivars, berry growth was more sensitive to preveraison water stress than to postveraison stress. Cultivar was the most significant factor influencing preveraison berry growth response, while year was the most significant factor affecting postveraison response. Maximum rates of sugar accumulation occurred for all cultivars between 75 and 85 days after anthesis (DAA) and were positively correlated with canopy percent shaded area measured at veraison. Sugar accumulation in most cultivars ceased by 110 DAA. Fruit phenolic composition was strongly influenced by cultivar, but preveraison water deficits increased anthocyanin concentration in most genotypes. The results suggest that berry growth and composition responses to water deficits are genotype dependent, and cultivar-specific irrigation protocols may need to be considered.

Funding Support: American Vineyard Foundation, California Institute of Water Resources

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Viticulture — Water Relations Session – CONTINUED

Effect of Postveraison Canopy Reduction and Deficit Irrigation on Merlot Grape Composition

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In several winegrowing regions of the world, climate change has initiated an acceleration of the ripening process, resulting in increased sugar concentration at harvest and in wines characterized by high alcohol levels, low acidity, and reduced varietal aroma. The limitation of photosynthetic carbon availability by drastically reducing canopy size has been proposed as a strategy to reduce berry sugar at harvest. However, in some cases, this strategy has negatively impacted other fruit composition parameters (e.g., anthocyanins). Our research investigated the effect of canopy manipulation on sugars and phenolic accumulation in the berries of grapevines subjected to different irrigation regimes. The trial was conducted at the experimental station of the University of Udine (Udine, Italy) on 28-year-old Merlot grapevines grafted on SO4 in 2011 and 2012. We tested the effect of a severe postveraison canopy reduction (-50% of total leaf area) in combination with deficit irrigation to reduce sugar concentration and enhance phenolic concentration in the berries at harvest. Four treatments were tested: 1) Canopy reduction under deficit irrigation (CR-WD), 2) Canopy reduction under full irrigation (CR-I), 3) No canopy reduction under deficit irrigation (C-WD), and 4) No canopy reduction under full irrigation (C-I). In 2011, CR-WD treatment resulted in berries with lower sugar concentration and higher anthocyanin concentration than the other treatments. Canopy reduction was also effective in reducing sugar without altering anthocyanin concentration under well-watered conditions. In 2012, however, canopy reduction did not reduce sugar concentration at harvest. Our data suggests that modifying the source-sink balance via canopy reduction is a viable practice for modulating sugar accumulation only when the leaf area/crop weight ratio is a limiting factor. Seasonal weather variability may overcome the targeted ratio by compensating fruit size, and/or yield and canopy reduction should be managed accordingly.

Funding Support: This research was partially funded by EU Cross-Border Cooperation Programme Italy-Slovenia 2007-2013 (VISO) and the Friuli-Venezia-Giulia Region (GiSVI)

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Viticulture — Water Relations Session – CONTINUED

Split-Container Culture Reveals Developmental Differences in Grapevine Roots Grown in Heterogeneous Soils

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Soil heterogeneity and root-shoot interactions confound the identification of root-specific traits that optimize and maintain water uptake in grapevines. The ambiguous roles of genetic versus environmental factors in determining root survivorship and development also confound predictions of mature root phenotype from observations of young roots. In the present study, we used split containers to observe root development in single root systems exposed to contrasting moisture regimes and with limited shoot stress. Plantlets derived from green cuttings of the drought tolerant rootstock 110R and the drought sensitive rootstock 101-14Mgt were pruned to two roots of approximately equal length and transplanted into split-container units consisting of two pots joined by a 1.6 cm compression elbow. Plants were watered to field capacity, and after one week of establishment, one pot in each unit was maintained at or near field capacity, while the other pot was allowed to dry to below 30% field capacity before rewatering. Controls included split containers in which both pots were well watered. After two drying cycles, roots were removed from pots and evaluated for fresh weight, dry weight, and architectural components, including length, diameter, and surface area. For both rootstocks, fresh and dry weights of roots grown in drying soil were significantly reduced compared with roots in wet soil, as were root architectural components. Increased dry:fresh weight ratios in roots grown in dry soils compared with those in moist soils were more pronounced in 101-14Mgt than in 110R, indicating more drastic morphological changes restricting hydraulic conductivity in the drought sensitive cultivar. Our results suggest that developmental responses to environmental factors in early growth are important in determining the ultimate form of grapevine root systems, and diminished response to dry conditions might be a key component in drought tolerance.

Funding Support: California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, American Vineyard Foundation, CDFA Improvement Advisory Board, California Table Grape Commission, Louis P. Martini Endowed Chair for Viticulture

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Nitrogen Symposium

Impact of Cultivar and Crop Load on Yeast Assimilable Nitrogen in Cider Apples Grown in Virginia

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Yeast assimilable nitrogen (YAN) is an essential yeast nutrient for wine and hard cider fermentation. Inadequate sources of YAN can result in stuck or sluggish fermentation and the concentration of individual amino acids can impact the formation of adverse fermentation products such as hydrogen sulfide. The objective of this study was to determine the YAN [primary amino nitrogen (PAN) + ammonium ion] concentration in fruit produced under low, medium, and high crop load conditions in the Ramey York apple (*Malus domestica*) and to survey YAN concentration and composition in 15 apple cultivars grown in Virginia with potential for use in hard cider production. Crop load was inversely proportional to the YAN content of Ramey York apples, with low crop load (73 mg/L N) having significantly higher YAN concentration as compared to medium (60 mg/L N) and high crop loads (57 mg/L N). PAN was also inversely correlated with crop load, but ammonium ion levels were not. Our survey of 15 cultivars grown under the same conditions indicated that YAN concentration was variable across cultivars, with the lowest average YAN content of 25 mg/L (Golden Delicious) and the highest average YAN content of 172 mg/L (Enterprise). In all cider apple varieties, the major component of total YAN was PAN (min: 18 mg/L, max: 165 mg/L), whereas the ammonium ion concentrations were consistently lower (min: 1 mg/L, max: 15 mg/L). The results of this survey indicate that YAN is highly variable across cultivars grown under the same management practices, location, and growing season, and also indicate the low levels of ammonium ions generally present in these cultivars. Further research is required to ascertain whether these patterns will be consistent in subsequent growing seasons.

Funding Support: Virginia Department of Agriculture and Consumer Services Specialty Crop Block Grants, 2013 – 2016 Virginia Agricultural Experiment Station (VAES) and the USDA-NIFA Hatch Program

Comparison of Yeast Performance between Dry and Liquid Strains at Three Different DAP Target Concentrations in Red Must

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Many variables contribute to yeast performance during fermentation. The first factor that will be explored in this experiment is nitrogen concentration. Nitrogen availability during fermentation constitutes a crucial role in yeast health and metabolism, wine stability, off flavor production, and fermentation

completion. The second factor will be comparing performance of yeast strains in liquid form versus dry form. In this study, liquid and dry yeast strains will be used in a side by side trial to ferment red must with three different diammonium phosphate target concentrations (150, 250, and 350mg/L). The focus will be to compare fermentation rates, effect on microbial stability, and overall aromatic profile differences between batches, in the hope that some light will be shed on potential differences in nitrogen requirements between dry and liquid forms of yeast strains. Liquid and dry forms of the following yeast strains will be used in the side by side trials: 71B, EC1118, ICV D254, Enoferm AMH, RC212, and Enoferm BDX. There will be eight fermentation batches for each yeast strain. Two of these batches will consist of a dry strain control and a liquid strain control containing no DAP additions. The other six will consist of a dry strain and liquid strain for each of the three DAP target concentrations. During alcoholic fermentation, batches will be monitored for rate of sugar consumption, pH, microbial stability, and hydrogen sulfide production. Postfermentation, residual sugar, and alcohol by volume will be determined for each batch, as well as a volatile compound profile by gas chromatograph.

Funding Support: White Labs, Inc.

Evaluation of Nitrogen Management Schemes for Intensively Cover-Cropped Vineyards

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Cover cropping is a nearly ubiquitous practice in vineyards of the eastern United States. The primary rationale behind the strategy is to moderate vine vigor, to minimize soil erosion, and to improve soil physical and biological features. However, cover cropping can lead to increased nutrient competition and have deleterious impacts on vine nitrogen status and yeast assimilable nitrogen (YAN) in fruit. This study evaluated the effect of nitrogen management schemes on chemical and physical characteristics of the vine, including canopy structure, leaf chlorophyll content, components of yield, and berry YAN. Differing rates of soil and foliar nitrogen fertilizer were applied to Sauvignon blanc, Petit Manseng (*Vitis vinifera*), and Vidal blanc (*Vitis* ssp.) grown in Virginia. One Petit Manseng experiment had the highest season-long chlorophyll content index (CCI) values and highest petiole nitrogen with a moderate rate of soil nitrogen applied at bloom (45 kg N/ha). In another Petit Manseng experiment, the highest berry YANs came from a split application of nitrogen immediately postveraison (15 kg N/ha). The season-long CCI values and leaf petiole nitrogen in the Sauvignon blanc vineyard were highest among the high soil nitrogen treatment (60 kg N/ha in split applications). The must titratable acidity was significantly higher than the control in the foliar treatment (30 kg

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N/ha over six sprays of urea starting at bloom). Under-trellis cover cropping with clover showed no significant differences in the Vidal blanc in the first year of treatments. Enhanced point quadrat analysis indicated no differences in general canopy architecture across all treatments and cultivars in the four experiments. To date, this study has identified nitrogen management schemes that have potential to effectively increase YAN and vine nitrogen status in cover-cropped vineyards, although further research is required to refine these management strategies and to evaluate potential effects over multiple years.

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

Effect of Foliar, Soil Nitrogen, and Sulfur Applications on Petit Manseng Grape Volatiles and Wine Aroma and Flavor

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The effect of foliar nitrogen and sulfur applications were evaluated as means to improve grape composition of *Vitis vinifera* cv. Petit Manseng vines over two seasons. Four treatments were applied annually in a randomized block design with six replicates of six vines each: control without fertilization, soil nitrogen at 30 kg/ha, foliar nitrogen at 15 kg/ha, and foliar applications of nitrogen plus sulfur at 15 kg/ha and 5 kg/ha, respectively. Fruit differed in ammonia, arginine, and yeast assimilable nitrogen concentrations in both seasons. Electronic nose measurements on field clusters and laboratory berry analyses demonstrated differences among treatments in volatile content. Solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) analysis identified 27 free and 52 bound juice compounds. Wine aroma and flavor profiles of Petit Manseng were evaluated using descriptive analysis. Analysis of variance (ANOVA) showed variation in 23 of the 24 attributes used. Wine principal component analysis (PCA) of aroma attributes showed 23.5% of the variation from PC1, while flavor by mouth and texture/mouthfeel attributes showed 26.3% of the variation due to PC1.

Funding Support: Virginia Wine Board and North Carolina Wine and Grape Council

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Nitrogen Symposium – CONTINUED

Effect of Nitrogen Application Rate and Timing on Methoxypyrazine Content of Okanagan Sauvignon blanc Grapes

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Concentrations of vegetative methoxypyrazine (MP) aromas can be high in grapes grown under shady, cool conditions and in immature fruit. High N status is one factor that can cause vigorous, unbalanced vines. Nitrogen is also a component in the structure MPs, and it is unknown if nitrogen directly influences MP biosynthesis. This research shows the effects of nitrogen application rate (40, 60, and 80 kg/ha) and timing (budbreak, fruit set, veraison) on MP metabolism, vine vigor, and fruit composition during maturation in Sauvignon blanc grapes. Fruit and canopy measurements were taken four (2011) or five times (2012) through development from pea size berries to mature fruit. Isobutylmethoxypyrazine and isopropylmethoxypyrazine were the predominant MPs of the five MPs measured using GC-NPD-SPME. Treatments with lower N rate at budbreak and higher N rate at fruit set resulted in high isobutylmethoxypyrazine throughout maturation and high isopropylmethoxypyrazine early season compared to treatments with higher N at budbreak and lower N at fruit set. Late N application at fruit set and veraison resulted in the lowest isobutylmethoxypyrazine at maturity. Isopropylmethoxypyrazine was present in trace amounts at maturity with no significant effects among treatments. Leaf total N and fruit yeast assimilable nitrogen content correlated with higher N application rates, and increases were measured 15 days after N application. There were transient differences among treatments for sugar and titratable acidity and no differences among treatments for canopy dimensions, including cluster exposure and fruit temperatures. Treatments with both moderate N rates at budbreak and higher N rates at fruit set resulted in the highest pyrazine content through development. This may be a direct effect of N on pyrazine accumulation since there were not apparent differences between treatments for canopy size, canopy density, fruit exposure, and fruit temperature.

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

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Nitrogen Symposium – CONTINUED

Balanced, Essential Metals for Yeast Growth and Metabolism in Fermentation Processes

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Metals are very important in several areas of yeast cell physiology. Yeast cells need metals for maintaining cell and organelle structural integrity, for cell-cell interactions such as flocculation, for gene expression, and for cell division and growth. Potassium, magnesium, calcium, and zinc are cationic nutrients that play essential structural and functional roles in yeast cells and are particularly significant in fermentation processes. Along with other substrates like fermentable carbohydrate, utilizable nitrogen, and vitamins, minerals provide essential factors for yeast growth. Unfortunately, the latter are often overlooked as important determinants of yeast fermentation performance, and it should be emphasized from the outset that the nature and concentration of metal ions supplied in growth media can have a significant impact on yeast-based industrial processes. After all, a prerequisite for the success of any yeast biotechnology is a thorough understanding of the factors that regulate nutrition, growth, stress responses, and metabolism in yeast cells. These include inorganic factors. Yeast cells require a wide range of metals for their growth and metabolic functions, and the mineral nutrition of yeasts is thus very important in ensuring successful fermentation, particularly in alcohol production processes. For ethanol fermentations, these ions include magnesium and zinc that act as cofactors for important fermentative enzymes (glycolysis and alcoholic fermentation, particular at higher alcohol levels) and also as modulators of environmental stress. Some metals inhibit yeast growth and metabolism, either by antagonism with essential metals (calcium against magnesium and zinc) or through direct toxicity effects (as with heavy metals). The special nutrient process (Erbsloeh F3 concept) takes into account the roles of magnesium, calcium, and zinc in the physiology of industrial strains of the yeast *Saccharomyces cerevisiae*.

Funding Support: Erbslöh Geisenheim AG

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Enology and Viticulture

Perception of Sauvignon blanc Aroma

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Odorant mixtures are experienced in two manners, by an analytical process (elemental) or by a synthetic process (configural). However, unlike the perception of mixtures of colored light, it appears that odorant mixtures can always be perceived analytically with sufficient training or experience. For example, one might hear, "this wine smells of passion fruit and bell peppers" (analysis) followed by, "it must be a Sauvignon blanc." Among the most potent odor active volatiles in Sauvignon blanc (SB) are 3-mercaptohexanol (3MH, smells like passion fruit), 2-isobutyl-3-methoxypyrazine (IBMP, smells of bell pepper), and 4-methyl-4-mercaptopentan-2-one (4MMP, smells like boxwood). This poster examines the perception of mixtures of these compounds in a model wine system using sniff olfactometry (SO). The SO is an experimental platform integrating Psychopy software and an olfactometer designed to deliver 15 mL headspace gas in 70 ms pulses to a subject viewing cues on a computer monitor. Subjects sniff mixtures of 3MH, IBMP, and 4MMP in different ratios to determine the interaction between these components in two types of experiments: 1) determine the ratio of each pair that completely masks one of the components, and 2) determine the composition of the tertiary mixture that best mimics a commercial SB wine. If we define the concentration ratio at which subjects choose one of two words, e.g., "passionfruit" or "boxwood," 50% of the time as a 1:1 potency ratio, then increasing one component 5-fold and decreasing the other by 5-fold yields a mixture with a 10:1 potency ratio. Generally, a 10:1 or 1:10 potency ratio is indistinguishable from a pure sample of the major component. Using two SB wines with distinctly different aromas for comparison, we report on the detectability of 3MH, IBMP, and 4MMP in binary and tertiary mixtures and speculate on the nature of the Sauvignon blanc odor image.

Funding Support: Analytical testing

Carrageenan Use in White Wine Fining: Features, Effectiveness, and Effects on Chemical Composition

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Traditionally, the protein stabilization process in white wines come through the use of bentonite. Despite being inexpensive and very effective in the removal of unstable proteins, bentonite leads to a strong aromatic and structural depletion of wines. Additionally, bentonite fining causes increased costs due to loss of product and wastewater treatment. In this work, the results of carra-

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Enology and Viticulture – CONTINUED

geenan use on wine are presented. Carrageenans are polysaccharides extracted from red seaweeds (*Chondrus crispus*), consisting of repeated units of galactose and 3,6 anhydrogalactose, alternatively linked by α 1-3 and β 1-4 glycosidic bonds. Carrageenans are characterized by one or more sulphonic groups ($-OSO_3H$): κ (kappa), ι (iota), and λ (lambda), with one, two, and three sulfonic groups, respectively. These groups determine the negative electrical charge and reactivity towards proteins. They are already used in the brewing industry due to their effectiveness in protein removal. In this research, electrical charge of various carrageenans (by titration technique with streaming current detector), protein removal capacity (by Codex OIV method using model wine solution), and wine stabilization power in comparison with bentonite are evaluated. Their activity was assessed against various protein fractions which are considered responsible for haze formation (thaumatine-like proteins and chitinases) and the required dosage for obtaining stability, also in combination with chitosan. In addition, the effects of protein stabilization with carrageenans on wine aromatic compounds were evaluated. Sensorial analysis showed that treatment with carrageenans is more respectful of wine qualitative characteristics. Finally, we compared the sediment amount and metal release of carrageenan and bentonite.

Funding Support: Università degli Studi di Verona Enologica Vason S.p.A.

Grapevine Leafroll and Red Blotch Diseases in Washington Vineyards

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Grapevine leafroll (GLD) and grapevine red blotch (GRBD) are considered important viral diseases affecting winegrapes (*Vitis vinifera*) in Washington vineyards. Previous studies have indicated that *Grapevine leafroll-associated virus 3* (GLRaV-3) is predominant among the GLRaVs documented in grapevines showing symptoms of, or suspected for, GLD. However, information on the distribution of GRBD and Grapevine red blotch-associated virus (GRBaV) in Washington vineyards is not available. Therefore, a survey was conducted during the 2014 season to gather data on the relative distribution of GLD and GRBD in vineyards. Vineyard blocks planted with red- and white-fruited winegrape cultivars in six AVAs in eastern Washington were surveyed for the presence of GLD and GRBD. In the case of red-fruited cultivars, leaf samples were collected from individual grapevines exhibiting typical symptoms of GLD and GRBD and from grapevines suspected for leafroll- or red blotch-like symptoms. Due to the absence of visual symptoms of GLD and GRBD in white-fruited cultivars, leaf samples were collected from individual grapevines randomly in the vineyard block. A total of 546 samples from 11 red-fruited and 30 samples from four white-fruited cultivars were collected and tested

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separately for GLRaV-3 and GRBaV by RT-PCR and PCR, respectively, with appropriate controls. The virus-specific DNA bands amplified from a select number of samples were cloned and sequenced for additional confirmation of GLRaV-3 and GRBaV. The results indicated presence of GLRaV-3 and GRBaV as single virus infections in ~82% of samples and both viruses as co-infections in ~18% of samples. Among the samples with single virus infections, ~71 and 29% tested positive for GLRaV-3 and GRBaV, respectively. Based on these results, it can be concluded that GLRaV-3 is predominant compared to GRBaV and co-infection of these two viruses are less frequent than single virus infections.

Funding Support: WSDA-Specialty Crop Block Grant Program (Project No. K1275), Washington State Commission on Pesticide Registration, WSDA Nursery Research Funding, Wine Advisory Committee of the Washington Wine Commission; Agricultural Research Center, WSU-CAHNRS, USAID-Indonesia, Altria-Chateau Ste. Michelle Wine Estates

Influence of Vineyard Floor Competition on Growth, Yield, and Berry Quality in Southeastern Vineyards

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Resurgence of interest in growing winegrapes in the southeastern United States has occurred in the last two decades. However, viticulture in this area of the country is challenging due to excessive vine vigor and significant disease pressure due to high precipitation during the growing season. Additionally, high humidity, excess shading, and high temperatures make achieving optimum fruit quality challenging. A study was conducted at a commercial vineyard in the Yadkin Valley region of North Carolina from 2011 to 2014 to determine effects of five vegetation-free strip widths and late-season weed competition on vine growth, berry quality, and yield of Cabernet franc cl. 312 on 101-14 MGT rootstock. The vineyard floor was sown to Kentucky 31 fescue after harvest in 2010. In spring 2011, vegetation-free strip widths (VFSW) of 0, 0.3, 0.6, 1.2, and 2.4 m were established beneath the vines using paraquat or glufosinate herbicides and managed continuously all four years. At the onset of veraison, herbicide application in half of each plot ceased and native vegetation was allowed to establish in these subplots to determine the effect of late-season weeds on vegetative growth and berry quality. Vegetative growth and berry quality were not affected by late-season weeds in the subplots. Summer fresh pruning weight was greater for wider VFSW in 2012 and 2014. Winter pruning weight decreased as VFSW decreased in 2011 and 2012. Lateral shoot number decreased as VFSW decreased in 2011, 2012, and 2013. Yield increased as VFSW increased in 2011, 2012, and 2013.

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Cluster weight was lowest in narrow VFSW in 2011 and 2012. In 2011 and 2014, Brix increased with decreasing VFSW. In 2011, titratable acidity decreased as VFSW decreased. These results suggest that the use of narrow VFSW (≤0.6 m) does reduce vegetative growth and improve berry quality measures.

Funding Support: USDA/NIFA Specialty Crop Research Initiative

Evolution of Pigmented Tannin

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As pigmented tannins in wine have demonstrated importance not only to persistent color but also to mouthfeel and texture, an investigation into the responsible molecules of pigmented tannins was undertaken. The analysis utilizes quadrupole time of flight, quadrupole trap, and Fourier transform ion cyclotron mass spectrometry. With methods developed for this project in previous years, the pigmented tannin fraction of young (2 years) and old (22 years) Cabernet Sauvignon wines have been examined for their pigmented tannin constituents. In addition, seven Cabernet Sauvignon wines spanning 35 years from a single vineyard have been examined by these methods to obtain semi-quantitative tracking of pigmented tannin evolution. Traditional tannin measurements of percent galloylation, mass recovery, and mean degree of polymerization, have been performed on these wines to correlate these new findings to previous reports and relate the MS data to current measurements in wine chemistry. Since many compounds were initially unknown, we pursued structure identification by MS fragmentation.

Funding Support: American Vineyard Foundation

Whole-Canopy Gas Exchange of Merlot Grapevine is Affected by Interaction of Crop Load and Irrigation

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Growers in the San Joaquin Valley of California are mechanizing vineyard operations. However, the ubiquitous California sprawl trellis (CS) and the procumbent habit of cultivars make this a challenge. This experiment was undertaken to investigate the effects of trellis conversion and applied water amounts on vegetative compensation, yield components, whole canopy photosynthesis, and phenolic composition of fruit and wine of Merlot/Freedom grapevine. The following factors, crop load systems, and applied water amounts were arranged factorially in a randomized complete block. Crop load systems included a spur-pruned CS trellis (HP), a cane-pruned CS trellis (CP), and a mechanically pruned single high-wire trellis (SHMP). Applied water amounts were sus-

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tained deficit irrigation (SDI), where vines were maintained at a midday leaf water potential (Ψ) of -1.2 MPa and were irrigated to 0.8 of crop evapotranspiration (ET_c) from budbreak until harvest, and regulated deficit irrigation (RDI) that received 0.8 of ET_c from budbreak to fruit set, whereafter 0.5 ET_c was replaced to maintain (Ψ) at -1.4 MPa until veraison, but not thereafter. The leaf layer of HP was 18 and 11% higher than that of SHMP and CP, respectively. The yields of HP and CP were 28 and 22% lower than that of SHMP. The phenolic compounds measured included anthocyanins, proanthocyanidins, flavonols, flavan-3-ols, and nonflavanoid compounds. A precocious and earlier exposed leaf area was produced by SHMP. Although CP displayed a greater whole canopy photosynthetic rate, when calculated on a leaf area basis, SHMP displayed the greatest leaf assimilation rate. Applied water amounts did not affect canopy architecture or yield efficiency. This project provides applied information about the optimum crop load management and deficit irrigation strategies for maintaining or increasing yield and phenolic composition during the transition between manual and mechanically managed vineyards in the hot climate.

Funding Support: American Vineyard Foundation

Effect of a No Plunge Winemaking Technique on Phenolic Extractions and Pigment Composition in Merlot Wines

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Phenolic extraction (anthocyanins, total phenolics, and tannins) and pigmented tannin evolution in Merlot wines were evaluated and compared over a period of one year for wines made using three different winemaking techniques. Traditional maceration (2X plunges per day), plunging during the second half of maceration only, and no plunging at all regimes were compared using triplicate 12 kg vinification. No prefermentation maceration occurred and a 15 day fermentation and postfermentation period was used. The wines were analyzed daily during the maceration period and regularly after pressing during the next 11 months. Phenolic, tannin, and color measurements were analyzed using UV-vis spectral readings processed through the Australian Wine Research Institute WineCloud. At time of pressing on day 15, total phenolics, total pigment, and free anthocyanins were significantly lower in concentrations in the traditional maceration treatment compared with no plunge. Tannin concentration was also significantly lower during days 10–12. Pigmented tannin was consistently at higher concentrations in the traditional maceration treatment during maceration, although not significantly so. The significant differences were maintained as the wine developed over the next 11 months. However, the no plunge trial wine displayed increased pigmented tannin development compared with the traditional maceration, being significantly higher in

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pigmented tannin from about day 90. Higher color and phenolic extraction in the no plunge trial led to greater stable color development in resulting wines. The alternative no plunge winemaking had an important and useful effect in modifying the pigment profile of the wines. General wine quality parameters did not differ significantly between the three treatments.

Funding Support: Eastern Institute of Technology

Volatile Composition of Iowa's Cold-Hardy Grape Wines with the Use of GC-MS and Electronic Nose

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Each wine has a unique set of volatile compounds that gives it a certain characteristic flavor. There are several factors contributing to this: grape variety, geographical origin, grapegrowing practices, fermentation conditions, as well as fining operations and aging. Among those parameters, grape variety affects flavor composition to the greatest extent in the resulting wine. Knowledge about volatile organic compounds composition makes it possible to relate the presence of certain compounds or group of compounds to the perceived flavor and indicate those responsible for distinctive characteristic of Chardonnay, Riesling, Cabernet Sauvignon, and Pinot noir wines. This research interest has grown due to current New Worlds' Producers emphasis on varietal labeling. Since *Vitis vinifera* has a very long growing tradition, their wines have been extensively studied. Although their flavor characteristic is somewhat predictable or known, flavor composition of new, cold-hardy varietal wines has not been sufficiently studied. Due to the fact that cold-hardy grape cultivars consist of two or more species, composition of wine produced from such grapes is distinctive and driven by each constituent. Therefore, the objective of this study was to characterize volatile composition of wines produced from three white cold-hardy grape varieties, LaCrosse, La Crescent, and Edelweiss. The conducted studies, based on applying modern extraction techniques such as SPME coupled with GC-MS and electronic nose, has enabled comprehensive characterization of cold-hardy grape wines and allowed distinguishing, on the basis of volatile fraction composition, the grape variety that was used for wine production.

Funding Support: Midwest Grape and Wine Industry Institute

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Cell Wall Polysaccharides Release during the Alcoholic Fermentation by *Schizosaccharomyces pombe*

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The addition of commercial products containing polysaccharides derived from yeast cell walls to wine is becoming a common practice during the winemaking process. The reduction in protein and tartrate instability, improvement of mouthfeel, increasing sweetness and roundness, decreases in astringency, prevention of tannin aggregation and precipitation, addition of complexity and aromatic persistence, stabilization of the color of red wines, and stability of the foam of sparkling wine have been reported as positive enological properties associated with polysaccharides use. An interesting alternative to the addition of these commercial exogenous polysaccharide-based products could be the use of yeasts able to release high quantities of these compounds during the alcoholic fermentation. Recent results have shown that non-*Saccharomyces* yeasts are generally characterized by the capacity to release high quantities of polysaccharides. However, few studies have considered the release of polysaccharides by *Schizosaccharomyces pombe*, a yeast also found in wine production. *S. pombe* strains have found application in winemaking mainly because of their ability to reduce malic acid in grape juice and/or wine. We evaluated the ability of six different yeast strains of *S. pombe* to release polysaccharides during the alcoholic fermentation. Interestingly, at the end of the alcoholic fermentation, all of the *S. pombe* strains released a quantity of polysaccharides three to four times higher than that released by a commercial *Saccharomyces* yeast strain under the same fermentative conditions of synthetic juice. Polysaccharides released during the alcoholic fermentation were analyzed using a combination of analytical techniques for carbohydrate composition, protein profile, and degree of polymerization of released N-glycans.

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

Rootstocks Affect the Hardiness and Survival of Young Grapevines Exposed to Lethal Winter Temperatures

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In the Pacific Northwest where phylloxera infestations are rare, own-rooted vines are often grown to allow for vine recovery through sucker retraining following severe cold injury, yet the effects of rootstocks on vine hardiness are

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unknown. Thus, the impacts of rootstock variety on the scion bud hardiness of young grapevines were examined for Merlot and Chardonnay separately at two sites in the Okanagan Valley, British Columbia over the 2013-2014 dormant period. For both varieties, rootstocks evaluated were 3309C, SO4, Riparia Gloire, 101-14, 110R, Ramsey, Scharzmann, and 5C. Also evaluated were own-rooted Merlot or Chardonnay. Bud hardiness was measured three times during the dormant season using differential thermal analysis to determine bud lethal temperature exotherm (LTE) of scion buds. During the autumn acclimation period in November, scion buds were hardier on vines grafted onto rootstocks than on own-rooted vines. The hardiness enhancement due to rootstock ranged from 1.6°C to 5.3°C for Merlot and from 0.9°C to 3.3°C for Chardonnay. In January, during the period of maximum hardiness, rootstock effects on bud hardiness were diminished in Merlot and no effects were detected in Chardonnay. In early February, minimum ambient temperatures were -21.5°C and -19.5°C at the Merlot and Chardonnay sites, respectively, and killed 80 and 50% of the vines. At both sites, the own-rooted vines had the lowest survival rate, and vines grafted on 101-14 survived better than those on 110R. In March, during bud deacclimation, no effects of rootstocks on hardiness were detected. This study is ongoing for 2014-2015 and the results to date (Feb) are similar to in 2013-2014. These results indicate that rootstocks can be used to reduce vine winter injury, in addition to controlling vigor and phylloxera and nematode infestations.

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

Identification of Genes Involved in Expression and Establishment of the [*GAR*+] Prion

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Previous research has revealed a *de novo* prion-based mechanism in yeast that can overcome glucose repression, enabling yeast to utilize alternative carbon sources in the presence of glucose. Accordingly, this prion is named [*GAR*$^+$], standing for resistant to glucose-associated repression. To further understand the mechanism underlying [*GAR*$^+$] expression and to establish the frequency of occurrence, speed of establishing [*GAR*$^+$] on glycerol glucosamine media (GGM) was investigated in a set of sequenced wine strains. Frequency of occurrence was defined as the terminal serial dilution displaying good growth on GGM, and the speed of establishment was defined as the number of days required to observe the level of maximal growth, controlling for strain differences in growth on glycerol as the sole carbon and energy source. The strains showed different levels of growth and frequencies of prion appearance. Since the genes involved in prion establishment are known, we compared sequences

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of these genes across the strains to determine if any alleles were associated with the rapid establishment phenotype. *RGT1* encodes a transcriptional factor that mediates expression of *HXT* genes based on a repression mechanism. Among all the strains analyzed, the ones with a much faster speed and higher frequency (EC1118, Vin13, Lavin QA23, VL3) have truncations in the *RGT1* gene. An intact copy of *RGT1* was transformed into these strains and their ability to establish the [*GAR*$^+$] prion was investigated. The high frequency of mutation of the *RGT1* gene in wine strains may enable these strains to undergo specific membrane adaptations, but not decrease glucose transport upon [*GAR*$^+$] establishment. This hypothesis will be tested by direct measurement of *HXT3* gene expression levels. The *HXT3* gene is a downstream target of repression by the wild type *RGT1* protein. Loss of this protein would lead to a loss of reduction in this key glucose transporter.

Funding Support: UCD Scholarships

Mycotoxin Potential in High-Risk American *Vitis vinifera* Vineyards and Wines

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Mycotoxins pose a serious worldwide threat to the safety of numerous food commodities. Red wine made from *Vitis vinifera* grapes is particularly prone to contamination from ochratoxin A, produced by black-spored *Aspergillus* spp. worldwide, and it was recently discovered that these species can also produce the mycotoxin fumonisin B_2. Although wine surveys in most regions of the world have determined that ochratoxin levels are below the limit expected to pose a serious health threat, it remains imperative to monitor toxin levels in poor vintages and in new production regions to ensure safety. Furthermore, mycotoxigenic fungi are associated with off-flavor volatile compounds, such as geosmin, in finished wine. Currently every state in the U.S. has a commercial winery; however, a wide-scale sampling of mycotoxins in domestic wines has not been conducted. The humid environment of the southeastern U.S. proves challenging to *V. vinifera* grapes due to opportunistic fungal growth and high disease pressure. To determine the mycotoxin potential in America's high-risk southeastern vineyards, cluster samples representing 10 grape varieties were collected from nine vineyards during the 2013 harvest and analyzed for mycotoxigenic fungi and mycotoxins. In addition to a few *Aspergillus* isolates, a large number of *Fusarium* spp. with the ability to produce fumonisins at levels comparable to *F. verticillioides*, a well-known fumonisin producer, were readily collected. Fumonisins B_1 and B_2 have been reported in wine in other regions of the world. Therefore, with a toxigenic mycoflora present in the vineyards, it is imperative to analyze southeastern American *V. vinifera* wines not only for ochratoxin A, but for fumonisins as well. To this end, over 200

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bottles of 100% southeastern U.S., red *V. vinifera* wines were collected during 2013-2015 and are being analyzed for mycotoxins using LC-MS/MS. These wines represent 19 red *V. vinifera* grape varieties grown across six states during vintages 2001-2013.

Funding Support: USDA-ARS-Toxicology and Mycotoxin Research Unit, University of Georgia, Southern Region Small Fruit Consortium, and American Wine Society Educational Foundation Scholarships

Addition Time of Exogenous Tannin for Optimal Retention in Hybrid Red Wines

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To effectively increase condensed tannin content in hybrid red wines, exogenous tannin must be added above the manufacturer recommended dosage of 50-400 mg/L. Will addition timing improve tannin retention as well? In 2013, wines were made from Marèchal Foch, Corot noir, and Cabernet franc to compare the retention of exogenous tannins in interspecific hybrids and *Vitis vinifera*. For each cultivar, a commercial exogenous tannin product containing ~38% condensed tannin was added at a rate of 800 mg/L. Additions were made at crush prior to yeast inoculation (Must+), after the completion of alcoholic fermentation (PA+), and after the completion of malolactic fermentation (PMLF+). To determine the fate of tannins in each wine, a mass balance was performed, tracking the loss and increase in tannin (by weight basis) during the winemaking process. After eight months of aging, the retention of exogenous tannins was calculated using the theoretical condensed tannin addition (304 mg/L) and tannin measured in each wine. Marèchal Foch wines had tannin retention percentages of 12, 26, and 59% (Must+, PA+, and PMLF+, respectively). Corot noir evinced higher retention rates at 27, 60, and 73%. Retention in Cabernet franc was comparable to Corot noir at 31, 56, and 80%. The progressive increase in retention suggests that adding tannin after alcoholic fermentation reduces the portion lost to sorption or precipitation during wine processing. Mass balance calculations also showed that five to ten times more tannin was lost in the lees from PA to PMLF than from PMLF to bottling, even with tannin added after malolactic fermentation was complete. This study suggests that in the hybrid red cultivars studied, later additions of exogenous tannin increase condensed tannin retention to levels comparable to that in *V. vinifera*.

Funding Support: USDA/NIFA Specialty Crops Research Initiative: Grape & Wine Quality Eastern U.S. Initiative

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Manipulation of Phloem Structure toward Revealing the Mechanism of SOUR Shrivel

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Grapes (*Vitis vinifera* L.) have been used as a model system for understanding ripening and ripening-related physiological disorders in other fleshy fruits, hence studies were undertaken to elucidate the mechanistic basis of a paradoxical ripening phenomenon of grape berries known as suppression of uniform ripening (SOUR) shrivel. Field-grown grapevines in commercial vineyards were phloem-girdled to simulate the incidence of SOUR shrivel disorder. Pieces of bark were removed from the peduncles, and on the canes just below and above the proximal cluster at veraison and after veraison. Thereafter, symptoms of SOUR shrivel were monitored throughout the growing season. The girdled clusters exhibited typical symptoms of SOUR shrivel, which included flaccid berries with reduced fruit quality attributes manifested as low sugar levels, poor color development, low pH, and high acidity. Despite the girdling, the canes functioned normally as in healthy vines by repairing the girdled-induced wound by forming callus, which restored the vascular functionality. This study demonstrated that the incidence of SOUR shrivel is strongly linked to phloem structure and influx during ripening. Hence, a greater and clearer understanding of the structure and functionality of the vascular pathways of healthy and afflicted vines (simulated) will guide us to target specific causal factors and subsequently aid in its regulation using management operations.

Funding Support: Senior Visiting Fellowship, University of Bologna, Italy

Sensory and Chemical Effects of Postfermentation Maceration in Merlot Wines

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Maceration describes a process whereby grape solid material is in contact with juice or wine. During winemaking, maceration allows for phenolic compounds to migrate into the juice/wine from the skins and seeds. We evaluated the effect of maceration length on perceived sensory and measured chemistry. Merlot grapes were crushed into eighteen 200-L fermentation vessels. During fermentation, one volume of juice was pumped over the cap three times daily. Three of the 18 fermentors were pressed at dryness as the control treatments. The remaining fermentors were allowed to continue maceration and then pressed in triplicate groups at the following intervals: 1, 2, 4, 6, and 8 weeks. During the maceration period, each fermentor was pumped over for five

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minutes, once per day. Descriptive analysis (DA) was used to describe the wine sensory profile, which was then related to malvidin-3-glucoside (m-3-g), protein perceptible tannin, polymeric pigment contents, tannin species distribution (UHPLC-qTOF MS), and volatile profiling (GC-MS). Aromas of red fruit and citrus/floral increased with up to 6 weeks of maceration. Bitterness, astringency, drying, and hotness also increased with maceration length. Increased tannin concentrations were measured between the control and 1 week of maceration, but no differences were found among treatments of 1 to 6 weeks. Week 8 had significantly higher tannin concentrations than all other treatments. Maceration length was also associated with decreasing m-3-g and polymeric pigment. The observed decrease in pigment concentration is likely due to adsorption of anthocyanin to grape pomace. The study allowed us to determine compositional and sensory differences due to extended maceration. This experiment has provided additional data on a specific winemaking practice.

Funding Support: American Vineyard Foundation, Stephen Sinclair Scott Endowment

Chemical and Sensory Effects of Three Cap Management Techniques as Compared to Postfermentation Maceration in Merlot

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Cap management is a generic term to describe the techniques that are used to mix the fermenting must. This mixing allows retained heat to escape and contact between the skins and the fermenting juice. The effect of five cap management treatments on the perceived sensory and chemical measurements was evaluated. Merlot was crushed into fifteen 200-L fermentation vessels. During fermentation, six of the 15 fermentors were pumped over at a rate of one volume, three times daily. At dryness, three were pressed (POC) and three underwent 8 week maceration (PO8), then pressed. The next six fermentors were fitted with a purpose built wire mesh to submerge the cap into the fermentation. At dryness, three were pressed (SUB) and three underwent an 8 week maceration (SUB8), then pressed. The remaining three fermentors were punched down three times daily, then pressed (PD). Descriptive analysis (DA) and temporal dominance of sensation (TDS) described the sensory profiles of the wines, which were then related to tannin and pigment concentration, the distribution of tannin species (UHPLC-qTOF MS), and volatile profiling (GC-MS). Treatments SUB8 and PO8 were found to have less intense aromas of pepper/spice, and SUB8 had more intense alcohol aroma. Maceration also increased the perception of hotness, drying, and astringency. The TDS results showed a similar temporal pattern for the POC and the PD, initial sour-

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ness followed by astringency peaking at expectoration followed by hot, then astringency. The PO8 treatment was unique in that the astringency gave way to a bitter finish. The three control wines had significantly less tannin than the macerated wines, but of the control wine, SUB had the greatest amount.

Funding Support: American Vineyard Foundation, Stephen Sinclair Scott Endowment

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Cold Soak Management by Selected *Metschnikowia pulcherrima* Yeast

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Cold soak is a common practice for red winemaking in Burgundy, with the goal of managing Pinot noir color and aromatic intensity. The increase of color is thought to be related to high addition rates of sulfur dioxide during cold soak, which can have drawbacks while the increase of fruit character is believed to be related to the microbial flora diversity. However, there are organisms coming in with the grapes, especially *Kloeckera apiculata* or *Hanseniaspora uvarum* yeast, which can overproduce acetic acid and ethyl acetate. In addition, *K. apiculata* exhibits good growth under cool temperature and SO_2 cold soak conditions. *Metschnikowia pulcherrima* yeast is also commonly found in grape must. A strain of this species was selected by the IFV, from an original collection of 500 strains isolated from Pinot noir in Burgundy region. This selected strain was found to resist SO_2 (more than 50 ppm in must) and to establish a significant population even at low cold soak temperatures. When an active dried yeast biomass of this selected *Metschnikowia* was inoculated early under typical cold soak conditions, it dominated the microbial soup by the end of cold soak and the resulting must did not exhibit any sensory deviations such as acetic acid and ethyl acetate. In comparison with a non-inoculated control must under the same conditions, the early inoculation of the must with the selected *Metschnikowia* inhibited the *Kloeckera* activity. It is proposed that the winemaking practice of inoculating a selected *Metschnikowia* biomass in Pinot noir must at the onset of cold soak limits the overproduction of spoilage compounds by out-competing potential spoilage organisms and results in an overall positive impact on the wine quality.

Funding Support: Lallemand Inc Casdar (French state organization)

Selection of a New Wine Lactic Acid Bacteria Starter Culture for Red and Rosé Wines

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The selection of a new wine lactic acid bacteria strain was carried out on a collection of more than 200 *Oenococcus oeni* strains isolated during malolactic fermentation in wines mainly from the Rosé de Provence region of France. The objective of the selection performed by the IFV microbiology laboratory in Beaune was to obtain a wine lactic acid bacteria strain suited to the produc-

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tion of fruit driven red and rosé wines. The selection criteria included the ability to show good malolactic activity under harsh environmental conditions for wine lactic acid bacteria strains, as well as contribution to fruit driven aromatic complexity, low production of volatile acidity, and minimal impact on red and rosé wine color stability. Three of the selected wine lactic acid bacteria strains were found to have the ability to withstand the rigors of being produced in freeze-dried form for direct inoculation into juice or wine. One strain showed very good malolactic activity and vitality under limiting conditions: malolactic fermentation was induced and achieved in rosé wines at pH ≥ 3.1 (13%v/v), and up to 17% v/v on alcohol when the other environmental conditions were not limiting. The ability to induce malolactic fermentation at temperatures as low as 14°C (52°F) in less than three weeks was also tested. The selected strain was further characterized and determined to be different from other natural wine lactic acid bacteria isolates, to have the unique ability to slowly degrade low levels of citric acid, resulting in very low volatile acidity and very little diacetyl production. Another unique property of this new strain is its delayed and slow ability to degrade acetaldehyde, which may impact positively on the color intensity of Pinot noir wines. This new wine lactic acid bacteria strain shows good fermentation performance and conformed to the goals of the sensory selection criteria.

Funding Support: Lallemand Inc Casdar (French state organization)

Severe Water Deficit Promotes Terpene Biosynthesis in White Grapes

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Grapes are grown without irrigation in several dry Mediterranean regions. In recent years, prolonged drought periods during the summer have made the cultivation of grapes challenging in viticultural areas where irrigation was traditionally not used to support grape production. As was the case of several areas in northeastern Italy, many vineyards started experiencing severe water deficits during the summer, associated with a reduction of yield and changes in fruit and wine composition. Several studies have reported a positive effect of water deficit on the concentration of fruit phenolics in red grape varieties, but limited information exists on the effect of water deficit on fruit composition in white grape varieties. This study was conducted in Udine, in northeastern Italy, and explored the effect of severe water deficit on fruit secondary metabolism in Friulano (also known as Sauvignonasse or Sauvignon vert) vines. Two irrigation treatments (irrigation at 100% ET_c, no-irrigation) were compared in two consecutive seasons. Each season, berries were collected at

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six developmental stages. The major berry phenolics, carotenoids, and volatile organic compounds (VOCs) were analyzed with UHPLC-MS/MS, HPLC-DAD, and SPME-GC-MS platforms, respectively. The berry transcriptome was also investigated at three developmental stages in one of the seasons using an RNA-sequencing platform. Water deficit affected fruit secondary metabolism, particularly in the season when the water deficit was more prolonged and severe. Despite transiently modulating the phenolic concentration during berry development, water deficit had a limited effect on the phenolic concentration of the berry at harvest. Water deficit affected berry carotenoids and VOCs at harvest. Remarkably, the concentration of berry monoterpenes was induced under severe water deficit. The analysis of the berry transcriptome indicated that water deficit induced the expression of many genes that regulate the synthesis of phenolics, carotenoids, and terpenoids in the grape berry.

Funding Support: Mipaaf (Italian Ministry of Agriculture, Agri-Food and Forest)

Comparison of Multivariate Regression Methods for the Analysis of Phenolics in Red Wine

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The rapid prediction and tracking of phenolics vital to quality wine production was achieved by recording the entire UV-visible spectra of 102 Cabernet Sauvignon red wine samples throughout fermentation. To compare predictive methods, principle components regression, partial least squares regression, partial robust M regression, and ridge regression were applied across all samples. To monitor the effect of buffer constitution and sample concentration on predictive power, all predictions made using the UV-visible spectra of each sample were calibrated with traditional Harbertson-Adams assays and modified traditional assays with dilutions in the following ratios: no dilution, 1:4, 1:9, 1:14, 1:19, and 1:24. For traditional assay modification, buffers using urea and triethanol amine (TEA) set to pH 7 or pH 8 rather than sodium dodecyl sulfate (SDS) were used. Ridge regression gave the most accurate predictions when the wine sample was mildly diluted with model wine, and the urea/TEA buffer set to pH 7 was used for calibration. The same comparison was also applied to 102 red wine samples made from Syrah. Only Cabernet Sauvignon fermentation data gave reliable predictions, which suggests that the optimal predictive model may be cultivar specific.

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

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Evidence for the Development of Uneven Skin Strain in Water Soaked Grape Berries Prior to Cracking

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Berry cracking is commonly understood based on a thin shell model, in which berry cracking is the result of a uniform flesh pressure against a homogeneous, thin skin. In order to study this process, the surface of crimson and flame seedless berries were marked with small paint deposits (dots) on the berry surface and then soaked in water to induce cracking. Berry swelling and cracking was monitored by digital photography taken every five minutes under a dissecting microscope. Image analysis (using imageJ software) was used to track the coordinates of selected dots, and the distance between specific pairs of dots was calculated and expressed as % skin strain. Based on the thin shell model, our hypothesis was that during soaking but prior to cracking, skin strain should increase uniformly over the berry surface. For berries which did not crack, or for noncracked regions of berries which did crack, skin strain was uniform and increased linearly over time, to a range of +0.2 to +0.6% after 13 to 16 hours of soaking. However, for regions of berries which exhibited cracking, skin strain in the vicinity of the crack was uneven and strongly nonlinear over time, increasing or decreasing through a range of -2 to +3%, prior to the occurrence of a visible crack. These results indicate that cracking is associated with not only localized strains but also with uneven strains, contrary to expectations based on the thin shell model. Uneven strain in the vicinity of a crack, but prior to its appearance, may indicate nonuniform skin thickness or uneven flesh pressure in that area, but there was no visible nonuniformity on any grape surface prior to testing. This method should be useful in understanding differences between varieties and/or cultural practices in berry cracking susceptibility.

Funding Support: University of California, Davis

Impact of Enzymatic Oxidation of α-Linolenic Acid and S-Glutathione as Precursors to 3-Mercaptohexan-1-ol Formation

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Sauvignon blanc wines have become an increasingly popular cultivar grown due to the diversity of aroma descriptors found, including green pepper, grassy, lime, and passion fruit. These aromas are contributed to wine from volatile thiols, which are responsible for giving the characteristic aroma profile of New Zealand

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style Sauvignon blanc. This study explores the development of an important aromatic thiol, 3-mercaptohexan-1-ol (3MH), responsible for passion fruit and citrus aromas. This experiment explores the effect of different processing treatments on a viable pathway for 3MH formation through the combination of controlled machine-harvesting simulation, microoxygenation, and supplemental additions to determine the impact on aromatic potential of California Sauvignon blanc. This was accomplished by the use of hand-harvested fruit, with an extended maceration after crush, to mimic machine harvesting, and the application of what are believed to be the important contributors to the thiol formation. These contributors are a fatty acid, α-linolenic acid, and S-glutathione, a naturally occurring antioxidant in grapes and wine. This study has been developed to explore the enzymatic oxidation of α-linolenic acid to (E)-2-hexenal, which will conjugate to glutathione, to form what is known as the direct precursor to 3MH, 3-S-glutathionylhexan-1-ol (G3MH). Production and concentration of 3-mercaptohexan-1-ol (3MH), along with various volatile aromatic thiols, will be determined by GC/MS in the finished wine.

Funding Support: UC Davis Viticulture & Enology Department

Changing Vine Leaf Area to Crop Load Ratio Affects Cool Climate Grown Pinot noir Vine Performance and Fruit Color Change

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The effect of vine leaf area relative to crop load was investigated in the cool climate growing region of Canterbury, New Zealand using Pinot noir (clone 10/5) VSP trained vines. Three weeks after fruit set, vine shoot lengths were standardized and leaf area (LA) adjusted to 100, 50, or 25%. Crop load was similarly adjusted, with treatments applied in a 4-replicate split plot design. Leaf gas exchange via porometer was measured on two dates and leaf photosynthetic rate on one date prior to harvest. Leaf chlorophyll concentration was estimated with a SPAD meter on four dates. Fruit color change was visually rated mid-veraison to estimate changes to the start of berry ripening. LA to CL ratios varied from 4 to 69, and significant differences in SPAD were found on all dates, with LA having a greater influence than CL. Vines with 25% LA had consistently greener leaves than those with 100%, with 100% CL having significantly higher values only at harvest. Leaf gas exchange was highest in the 50% LA 100% CL treatment and lowest in the 25% LA 50% CL on both dates, with photosynthesis rate having the same results on the single date it was measured. Progression of fruit coloration varied between treatments, with those vines having greater LA progressing more quickly than those with less. Berry weight was reduced with lesser LA, but not affected by CL. Vine root

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carbohydrate measured in the dormant season showed a significant effect of LA, with 25% LA resulting in the lowest values. The results suggest that vines are able to compensate for variations in LA to CL ratio, but reducing LA to 25% of normal had negative impacts on vine CHO reserves and fruit development.

Funding Support: Lincoln University

Changing Vine Leaf Area to Crop Load Ratio Affects Cool Climate Grown Pinot noir Grape and Wine Composition

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The effect of vine leaf area (LA) relative to crop load (CL) was investigated in the cool climate growing region of Canterbury, New Zealand, using Pinot noir (clone 10/5) VSP trained vines. Three weeks after fruit set, vine shoot lengths were standardized and LA adjusted to 100, 50, or 25%. CL was similarly adjusted, with treatments applied in a 4-replicate split plot design. Grapes were harvested on 4 April, 2014, and replicates 1 and 4 and 2 and 3 were combined to make two wines from each treatment. Monomeric wine phenolics were quantified by HPLC. Both LA and CL had an effect on fruit Brix, with lower LA and higher CL associated with lower Brix. Only LA had an effect on titratable acidity, with 100% LA having a higher value than the other treatments. Neither LA nor CL had an effect on juice pH. CL had no effect on fruit YAN, but 25% LA had the higher values, expressed through primary amino acids rather than the ammonia component. No significant differences in juice organic acids, K+, or phosphorus were found. CL had significant effects on a range of wine phenolics, including caftaric acid, *p*-coumaric acid, rutin, and vanillic acid, while LA had effects on epicatechin, ferulic acid, protocatechuic acid, *p*-coumaric acid, and quercetin. LA to CL ratio influenced fruit and wine composition, mostly through extreme reductions of LA, though altering CL also had some effects.

Funding Support: Lincoln University

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A Comparison of Three Score Sheets Used to Evaluate Wine Quality in Wine Judging Competitions

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Different wine judging competitions use different score sheets to determine wine quality. We compared quality scores for Davis 20-point (UCD), OIV, and modified OIV (MV) score sheets. Nine Californian Cabernet Sauvignon wines were evaluated. Thirty-two wine experts were randomly assigned to use one of the score sheets. Results were analyzed by analysis of variance and correlation analyses. Strikingly, the UCD quality scores were incredibly low, ranging between eight and nearly 15. The OIV and the MV quality scores ranged from 64 to 81 and from 66 to 76, respectively. Both the OIV and the UCD quality scores discriminated among the wines but the MV scores did not. The quality scores were all significantly correlated across score sheets. Some MV descriptive attributes (MVDA) and some trained descriptive analysis (DA) attributes were significantly different across the wines. There were significant correlations between MVDA and DA scores for MVDA dried fruit and DA dried fruit, MVDA dried fruit and DA soy sauce, MVDA dark fruit and DA red fruit, MVDA dark fruit and DA canned vegetable, MVDA dark fruit and DA sulfur, MVDA sweet and DA sweet, MVDA bitter and DA astringent, MVDA astringent and DA astringent, MVDA astringent and DA hot mouthfeel, and lastly, MVDA potential with DA chemical. From these results, it would seem that even though the MV quality scores were not significantly different, the expert judges did separate the wines similarly to the trained DA panel when using the sensory attribute section of this score sheet. Additionally, the UCD and OIV score sheets did significantly discriminate among the wines. It is clear that this project only looked at the tip of the iceberg when it comes to wine competition score sheets, and future work is needed to study how these scores could be given more reliably and consistently.

Funding Support: American Vineyard Foundation

Determining the Impact of Cluster Thinning and Cluster Zone Leaf Removal on the Hormone Content of Pinot noir Grape Berry

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Understanding the mechanisms involved in the control of grape berry development is a major objective of modern viticulture, in order to produce optimally ripe crops with desirable characteristics. Field studies have demonstrated the influence of hormones in promoting berry growth and ripening. Recent stud-

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ies even suggest the implications of hormones late in the season during the fruit maturity. Apart from a developmental program, plant growth regulators are also responsible for the integration of cues like light, temperature, and plant water status, enabling the plant to adaptively respond to environmental changes. Although major progress has been achieved in measuring plant hormones, the natural biological variability existing in a grape cluster prevents drawing definite conclusions with respect to their dynamics during the grape berry development. It is assumed that individual berries following similar developmental progression over the season may follow the same hormone dynamics but these dynamics may be shifted in time from one berry to another. This is due to the unequal development of berries assumed to originate from unequal flowering/fruit set time during bloom. By developing a flower-to-berry tracking system, we were able to monitor the berry progression within a population of individuals that developed from similar flowering time. Having this major hurdle (uneven flowering time) addressed, the objectives of our experiment were: 1) to map the dynamics of eight families of plant hormones (Auxin, ABA, Cytokinins, GAs, Brassinosteroids, Jasmonate, Salycilate, and Ethylene) during the season in similarly developing grape berries, and 2) to evaluate the impact of two viticulture practices (cluster thinning and leaf removal) on the dynamics of these hormones. Interestingly, our preliminary results suggest that how advanced a berry is in its development is better explained by the volume occupied by the seed(s) rather than by its flowering time. Hormone quantification is in process to understand this particular trait we observed.

Funding Support: Oregon Wine Board - 2015-1662

Can Natural Sulfite Formation by Wine Yeasts Substitute for Sulfite Additions Disallowed in Organic Wine Production?

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U.S. standards for making organic wine currently prohibit winemakers from adding sulfites during processing. This prohibition does not apply to organic wines produced in the European Union, which puts U.S. winemakers entering this growing market at a distinct disadvantage. Because U.S. regulations do not forbid the presence of naturally-occurring sulfites, we are evaluating the possibility that certain commercial wine yeast strains may produce a sufficient amount of sulfite during fermentation to substitute for the additions normally made by winemakers. While most wine strains of *Saccharomyces cerevisiae* produce some sulfites during fermentation, the environmental and genetic factors that control production are not known well enough to be predictable. This study is determining such factors for a limited number of commercial strains that are known or presumed to produce higher than average levels of sulfite.

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The initial application is for production of white wines that do not undergo the malolactic fermentation or postfermentation aging. We are analyzing factors that control sulfite formation, utilization, and excretion. Potential winemaker interventions that could increase sulfite production but which are otherwise compatible with accepted winemaking practices and U.S. standards for organic wine production will also be explored. Two potential complications are getting specific attention. One is the risk of high levels of hydrogen sulfide formation in a strain that produces high levels of sulfite. The other is the recognition that winemakers typically choose starter cultures for reasons other than sulfite production and therefore, high levels of sulfite production cannot compromise other desirable characteristics expected in a commercial wine yeast.

Funding Support: USDA (Pacific NW Center for Small Fruits Research Program)

Genetic Analysis of Dormant Rooting Potential in *Vitis aestivalis*-derived Norton Grape

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Norton reportedly offers an abundance of traits, including cold hardiness and fungal pathogen resistance, which can be used to naturally improve existing *Vitis vinifera* germplasm. These traits are multifaceted; they decrease crop loss, vineyard management, and pesticides requirements, while maintaining enological quality via rot prevention, ultimately increasing the profitability of a vineyard or winery. Rooting inhibition of dormant cuttings, unfortunately, is reminiscent in Norton derived populations, and could potentially impede the commercialization of otherwise superior hybrids. To study rooting ability, a mapping population was developed in 2005 from a cross between *V. aestivalis*-derived Norton and *V. vinifera* Cabernet Sauvignon, resulting in 95 hybrid progeny, which was further expanded to 182 individuals in 2011. Previously in 2014, 87 F_1 genotypes from 2005 showed various degrees of rooting ability. Dormant cuttings of 133 F_1 genotypes from the entire mapping population are currently under test. The ultimate goal of this project is to study the dormant vegetative propagation capacity of Norton grape and to determine whether it can be improved in the F_1 hybrid population.

Funding Support: Missouri Department of Agriculture Specialty Crop Block Grant 14-SCBGP-MO-0029

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Characteristics of Polyphenol Adsorption Properties toward Grape Skin Insoluble Polysaccharides in Muscat Bailey A Wine

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Muscat Bailey A (Muscat Hamburg × Bailey, MBA) is native to Japan and the most popular grape variety for red wine in Japan. However, during maceration, the red color of MBA wine tends to fade markedly compared with that of wines made of *Vitis vinifera* grapes, such as Cabernet Sauvignon. One reason could be that MBA anthocyanins adsorb to insoluble polysaccharides in grape skin during maceration. To elucidate the effect of the anthocyanin adsorption to insoluble polysaccharides, we carried out a model experiment and found that ~20% of MBA anthocyanins adsorbed to insoluble polysaccharides in grape skin. Furthermore, anthocyanins having higher hydrophobicity showed greater adsorption to the insoluble polysaccharides. We also conducted small scale fermentation tests and analyzed anthocyanins in MBA must and skin. Anthocyanins having lower hydrophobicity were extracted faster into MBA wine than more hydrophobic anthocyanins. Anthocyanin content in grape skin in the must decreased continuously during maceration and never increased.

Funding Support: Grant-in-Aid for Scientific Research

Assessment of Winegrape Cultivars in North Carolina

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In a trial, 11 red winegrape (*Vitis vinifera)* cultivars/rootstock combinations were assessed for viticultural performance in Dobson, North Carolina. The plot was established in 2008 as a randomized complete block design with six replicates. Vine yield, fruit composition, and vigor were measured from 2010 to 2014. Cold damage to buds and 1-year-old canes was assessed for the 2013/2014 winter. Average growing degree days for the site were 3,814 with an average of 137 cm (54 in) of precipitation. Cultivars varied in yield (0.90 to 4.59 kg/vine), vegetative vigor (0.21 to 2.30 yield/pruning weight), soluble solids concentration (17.8 to 21.9), titratable acidity (3.8 to 7.2), and pH (3.44 to 3.91). The cultivars Carmenere and Nebbiolo had unacceptably low yields. Carmenere, Grignolino, Nebbiolo, Tinta Cao, and Touriga Nacionale had the highest soluble sugar concentration at harvest. Aglianico, Grignolino, and Nebbiolo had the most desirable titratable acidity and pH. Toruiga Nacionale and Tinta Cao were most affected by the cold and Lemberger was least affected. Results from this study can help inform cultivar selection for similar growing climates.

Funding Support: USDA/NIFA-SCRI and NC Wine & Grape Council

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Precursors of Hydrogen Sulfide during Wine Storage—the Role of Elemental Sulfur Pesticide Residues

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Hydrogen sulfide (H_2S) is frequently found in faulted wines with sulfurous off-aromas. H_2S is reported to increase during bottle storage of some wines, and the identity of all potential precursors responsible for this latent H_2S is still not resolved. We demonstrate that elemental sulfur residues (S-0) on grapes can not only produce H_2S during fermentation, but can yield wine soluble intermediates capable of generating more H_2S during storage. Grape juice was fermented to dryness in the presence of varying levels of S-0 (0-100 mg/L). The resulting wines were racked and sparged to remove H_2S, bottled under nitrogen, and stored reductively. After three months of storage, free H_2S increased proportionally to the original S-0 concentration ($p < 0.05$), corresponding to the conversion of ~1% of the original S-0. An assay for latent H_2S precursors based on (2-carboxyethyl) phosphine (TCEP) reduction was developed. Following solvent fractionation, latent H_2S precursors in wine derived from residual grape S-0 were found only in the aqueous phase. Ongoing work is focused on identification of the latent H_2S precursor by ESI-MS.

Funding Support: Northeast SARE

Cultivar Evaluation Study of Select *Vitis vinifera* Grapevines

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Due to their continued market dominance, there is interest in growing viable *Vitis vinifera* cultivars in challenging climates such as Missouri. A trial including cultivars that have demonstrated success in similar hot summer, cold winter environments was established in 2007 near Ste. Genevieve, MO. The vineyard was established with replicated cultivar blocks, planted at 8 × 10.5' spacing (vine × row) and trained to VSP with all 29 cultivars (17 red, 12 white) grafted to 3309C rootstock. The vineyard was maintained using common commercial practices. Data were collected from 2010-2012 including elements of yield, fruit quality, vine growth and bud mortality. Most cultivars within the study grew well vegetatively and produced similar yields. Notable exceptions were Malvasia Bianca and Muscat blanc, which produced low yield after suffering bud mortality at -18°C; Pinot noir, which consistently produced low yield; and Lemberger, which produced annually-variable yields of clean fruit. Petit Manseng and Petit Verdot consistently performed well for both viticultural and berry quality metrics. Petit Manseng demonstrated low bud mortality of 7%, pruning weight of 1.14 kg/vine, and fruit with a mean of 26.2 Brix, 3.4 pH, and 6.84 g/L titratable

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acidity (TA), while Petit Verdot also showed good winter hardiness and produced fruit with 23.9 Brix, 3.7 pH, and 5.11 g/L TA. The white cultivars Viognier and Semillon and red cultivars Cabernet franc, Teroldego Clendenon, and Tannat demonstrated individual strengths and generally delivered a reasonable balance of performance in yield, viticultural, and berry quality metrics. Tocai Fruiliano and Pinot noir, however, consistently failed to maintain adequate TA until harvest and subsequently produced poor quality fruit with respective TA and pH values of 3.10 g/L and 4.0 and 4.04 g/L and 4.0.

Funding Support: Missouri Wine and Grape Board and Viticulture Consortium-East

Antioxidant Properties of Blackberry Wine Produced Using Korean Traditional Winemaking Techniques

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Blackberries contain relatively high concentrations of anthocyanins and other phenolic compounds and thus are a good source of antioxidants in the diet. This research was designed to evaluate the antioxidant properties of wines made from Natchez and Triple Crown blackberries grown in Oklahoma, to quantify the major phenolic compounds in the wines, and to correlate antioxidant capacity with phenolic concentrations. Wines were produced using a modified traditional Korean technique that employed relatively oxygen permeable earthenware fermentation vessels. The fermentation variables were temperature (21.6°C vs. 26.6°C) and yeast inoculation vs. wild-type fermentation. The phenolic properties were measured using the Harbertson-Adams assay and antioxidant capacity was measured using the oxygen radical absorbance capacity (ORAC) assay. Based on the Harbertson-Adams assay, Natchez wines had higher contents of total phenolics, tannins, and anthocyanins than Triple Crown wines. In addition, wines produced using the higher fermentation temperature had higher total phenolic contents, nontannin pigments, and tannins than wines produced at the lower fermentation temperature. As expected, statistically significant positive correlations were observed between total phenolics, anthocyanins, nontannin pigment, and ORAC values. Interestingly, results suggested that a higher fermentation temperature at the start of the winemaking process followed by the use of a lower fermentation/storage temperature during aging may maximize phenolic compound extraction/retention. Overall, the winemaking technique used in this study produced wines high in antioxidant capacity compared to levels reported previously in the literature, which suggests that the Korean winemaking process could produce wines with excellent nutraceutical properties.

Funding Support: William McGlynn from Robert M. Kerr food and agricultural product center, Oklahoma State University

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Shoot Attributes of *Vitis vinifera* Converted from Cordon-Training and Spur-Pruning to Head-Training and Cane-Pruning

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The U.S. mid-Atlantic region is a humid environment and vines can grow in excess of their allocated trellis space. Some mid-Atlantic growers have designed new vineyards to utilize head-training and cane-pruning (cane-pruned) rather than cordon-training and spur-pruning (CT/SP), the more conventional system for this area. Anticipated gains in fruit quality and vineyard labor efficiency are drivers for vineyard conversion to new training techniques. We conducted an experiment with mature, cordon-trained vines to determine if conversion to cane-pruning was justifiable from the standpoint of vegetative growth suppression, maintenance of crop yield, and uniformity of crop ripeness. Seven-year-old (CT/SP) Cabernet Sauvignon (clone #337) grapevines were compared to similar vines that were converted to cane-pruning (cane-pruned). Vine spacing was 2.7 m × 1.5m. Reductions in vine size (dormant pruning weight 19% lighter for cane-pruned vines) and the higher shoot fruitfulness for cane-pruned vines compared to CT/SP vines were reported last year. Shoot size appeared to be more variable with cane-pruned vines compared to CT/SP vines by visual assessment in 2013. Individual shoots were tagged at veraison in the 2014 season from four cane-pruned and four CT/SP vines. Components of yield and primary fruit chemistry were assessed for these individual shoots at harvest. Dormant pruning weights were collected individually by cane. Cane-pruned vines had lighter canes than CT/SP; however, the variability of cane pruning weight was similar for the two pruning systems. Soluble solids at harvest had the same mean value, but greater variability for cane-pruned vines than for CT/SP vines. Converting mature, CT/SP vines back to head-training and cane-pruning is feasible; however, considerations must be given to in-row vine spacing and uniformity of crop maturity.

Funding Support: USDA NIFA SCRI

Impact of Elemental Sulfur and Nitrogen on Volatile Sulfur Compound Formation during and after Alcoholic Fermentation

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The influence of yeast assimilable nitrogen (YAN) content and elemental sulfur on the formation of volatile sulfur compounds (VSCs) during and after fermentation were investigated. A synthetic grape juice was utilized where the concentration and composition of YAN was adjusted. High (346 mg/L) and

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low (112 mg/L) YAN treatments were prepared where nitrogen was derived from primary amino acids, while a third treatment was prepared where the majority of the 346 mg/L YAN was derived from di-ammonium phosphate (DAP). Fermentations were performed by either a high H2S producing *Saccharomyces cerevisiae* strain (UCD522) or a non-H2S producing *S. cerevisiae* strain (P1Y2). H2S production was monitored with lead acetate tubes during fermentation and wines were assessed for additional VSCs by GC-PFPD. Variation in YAN concentration and composition impacted H2S production during fermentation and concentrations of VSCs in the wine. In particular, treatments fermented by UCD522 with high DAP had increased formation of H2S late in fermentation and significantly more methyl thioacetate in the wine postfermentation. The role of elemental sulfur in the formation of H2S and other VSCs postfermentation was also investigated. Pinot noir fermentations were performed where an addition of 0, 5, or 15 mg/g elemental sulfur was made. Fermentations were conducted by UCD522 or P1Y2 at 27°C and H_2S production measured using lead acetate tubes. Wines were assessed for additional VSC by GC-PFPD. Addition of elemental sulfur resulted in H_2S formation during the alcoholic fermentation independent of which yeast strain was used. H_2S production was higher in fermentations performed by UCD522 with increasing amounts of elemental sulfur resulting in increased production of H_2S. In addition, higher elemental sulfur additions also resulted in higher H_2S production late in fermentation (<1 Brix). Higher elemental sulfur also resulted in wines containing higher concentrations of methyl thioacetate postfermentation.

Funding Support: Oregon Wine Board

Impact of Wine Lees Levels and Composition on Formation of Volatile Sulfur Compounds during Aging of Pinot noir Wine

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Development of volatile sulfur compounds (VSCs) postfermentation can be a significant issue during both red and white winemaking. Unfortunately, our understanding of contributing factors or conditions that impact VSCs is limited, due in part to the complexity of their formation. This study focused on the development of VSCs in Pinot noir during postfermentation aging and the impact of wine lees levels and composition. Pinot noir wine was produced from grapes harvested from the Oregon State University vineyard. Fermentations were performed using two different *Saccharomyces cerevisiae* strains: RC212, a commonly used strain used for Pinot noir, or P1Y2, a non-H_2S producing strain. After pressing, wines were settled for 0, 24, or 72 hours before being dispensed into 12-L carboys. This resulted in wines with high, medium,

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or low amounts of lees. Wines were stored at 13°C and sampled after 2, 4, 8, 12, 24, and 36 weeks. Samples were analyzed for VSCs by GC-PFPD and for amino acids by HPLC-DAD. Concentrations of methionine and cysteine increased during aging and were influenced by yeast strain and level of wine lees. Wines produced using P1Y2 contained higher concentrations of methionine and cysteine than wines produced by RC212 at each level of wine lees. Higher lees levels also resulted in higher amounts of cysteine and methionine in the wines. Increasing levels of methionine and cysteine did not necessarily result in increased amounts of VSCs in the wines. In general, the concentrations of VSCs in the wines were below sensory thresholds. Dimethyl sulfide increased for all treatments during aging and was significantly higher in wines with heavy lees. Concentrations of methyl thioacetate and methionol were higher in wines produced by RC212 and changed little over time. Hydrogen sulfide increased in most wines between 2 and 4 weeks aging and decreased thereafter.

Funding Support: Oregon Wine Board

Characterization of Grape Apoplasmic β-1,3-Glucanase and Its Ability to Protect Against Fungal Diseases

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We report the characterization of grape apoplasmic β-1,3-glucanase *VvGHF17*. *VvGHF17* is one of the major proteins secreted into the culture medium by grape cell cultures. *VvGFH17* is expressed constitutively in grape leaves and berry pulp and skin. Experiments using *VvGHF17*:GFP fusion protein demonstrated that *VvGHF17* is localized at the apoplasmic space of cells, suggesting that *VvGHF17* is apoplasmic β-1,3-glucanase. *VvGHF17*-expressing *Arabidopsis* plants exhibited disease resistance to both *Botrytis cinerea* and *Colletotrichum higginsianum*, but not to *Pseudomonas syringae* pv. *tomato* DC3000. The inhibitory effect of *VvGHF17* on the infection by the fungi was promoted when *VvGHF17* protein was expressed in large amounts in the plants. In conclusion, *VvGHF17* may be a candidate for the transgene to create transgenic grapevines that are robust to simultaneous multiple fungal attacks.

Funding Support: University of Yamanashi

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A Mechanistic Investigation of Copper-Mediated Oxidation of Thiols in Model Wine

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Reductive sulfidic off-odors are characterized as egg-like, sewage, and burnt rubber, and as such are detrimental to the quality of wines. To control these odors, winemakers commonly utilize Cu(II) fining for their removal; however, the overall effectiveness of Cu(II) fining remains unknown. The process may lead to numerous undesirable outcomes, which include the loss of varietal thiol aroma, metal-catalyzed wine oxidation, and the accumulation of reductive sulfidic odors during storage. This study aims to understand the underlying mechanism by which copper interacts with various thiol compounds in wine. In addition to hydrogen sulfide (H_2S), 6-mercapto-1-hexanol was used to represent primary thiols and 3-mercapto-1-hexanol, a varietal thiol, to represent secondary thiols. Free thiol, oxygen, and acetaldehyde concentrations were monitored, as well as Cu-thiol complex formation in model wine. The study suggests that when free thiols are present in excess to Cu(II), a 2:1 thiol:Cu complex is quickly formed which results in H_2S and thiol oxidation. Cu(II) is reduced to Cu(I), which in turn reduces oxygen to hydrogen peroxide. As a result, ethanol is oxidized to acetaldehyde. It appears that H_2S is rapidly oxidized rather than forming insoluble CuS. The relative rates of these reactions will be shown and the extent to which H_2S can be selectively removed in the presence of thiols will be discussed.

Funding Support: Graduate Competitive Grant Program, College of Agricultural Sciences, Pennsylvania State University; Pennsylvania Wine Marketing & Research Board, Pennsylvania Department of Agriculture

Preharvest Restored Irrigation after Severe Water Deficit: Effect on Merlot Grapes and Wines

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Deficit irrigation strategies are commonly used on red grape varieties to improve important fruit and wine quality traits such as color and aroma compounds. However, when severe water deficit (WD) is maintained until harvest, it reduces berry weight and vine yield, and may increase berry sugar concentration, leading to wines with high alcohol levels. Our work tested a regulated deficit irrigation (RDI) strategy, that consisted in withholding irrigation from 30 days after anthesis to 30 days after veraison and restoring it during late ripening, with the aim to promote anthocyanin accumulation in the berry while preventing yield

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loss and excessive sugar accumulation at harvest. The experiment was carried out at the University of Udine (Italy), experimental station A. Servadei, in 2013. Irrigation was controlled with a subsurface irrigation system and rainfall was excluded with a tunnel structure roofed with an EVA film encompassing all the vine experimental rows. Grapevines were irrigated to 100% ET_c until berry set. Irrigation was then managed to maintain Ψ_{stem} between -1.2 to -1.5 MPa until 30 days after veraison, when irrigation was partially restored and managed to maintain Ψ_{stem} around -0.8 MPa. Control plots (C) were irrigated 100% ET_c for the entire season. At harvest, WD increased anthocyanin and tannin concentration in the berry compared to C vines, while no differences between treatments were observed in berry size, vine yield, and berry sugar concentration. Wines reflected berry composition and WD wines showed enhanced color intensity. Irrigation after veraison is commonly considered to decrease fruit quality because of the "dilution effect," which is expected to decrease the concentration of TSS and organic acids. Our results proved that severe deficit irrigation applied preveraison and released in the final part of the season avoided berry shrinkage and yield losses while improving the anthocyanin and tannin concentration of the fruit and wine.

Funding Support: EU Cross-Border Cooperation Programme Italy-Slovenia 2007-2013 (VISO)

Water Deficit Accelerates Veraison in Merlot Grapes

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The effect of water deficit on grape berry composition during ripening has been widely studied. Previous research has shown that water deficit increases the accumulation of several ripening related compounds and might accelerate the ripening process. However, few attempts have been made to determine the impact of water deficit on the onset of fruit ripening, and particularly on the veraison date (based on the change of berry color). Here we present the results of three years of observations (2011-2013) of the veraison process, and specifically of the changes of berry color from green to red, in Merlot vines under water deficit (WD) and well-watered (C) conditions. Irrigation treatments were established from 30 days after anthesis. A set of 40 clusters per treatment and five berries per cluster were randomly selected and tagged early in the season (pea-size stage). Each tagged berry was observed every two days and classified as green or not green (not green color indicated berries with pink, red, or purple color) according to its skin color. The day when the berry was classified as not green for the first time was recorded as the veraison date for that berry. Water deficit imposed before veraison accelerated the onset of veraison and reduced the number of days from when the first color changes

were observed to when all the berries had changed color (WD completed color change one week before C). In 2012, tagged berries were individually sampled and analyzed for their sugar content at harvest. No relationship between the time when the berry changed color and its sugar level at harvest was found in WD vines, while a linear relationship was observed in C vines with berries that changed color earlier having higher sugar concentrations.

Funding Support: Partially funded by EU Cross-Border Cooperation Programme Italy-Slovenia 2007-2013 (VISO) and the Friuli-Venezia-Giulia Region (GiSVI)

Grape Washing: Effects on Pesticides, Metals, and Fermentation Kinetics

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The principle of the French paradox is that moderate wine consumption has health benefits and now more than ever, the impact of pollution on food production is a matter of considerable relevance. Pesticides are used in agriculture to preserve the integrity of the final product, in order to protect the product from main diseases. However, their excessive use in past years (in developing countries they are still frequently used) has led to health problems and to environmental pollution. In particular, if used in concentrations which exceed threshold limit values, these synthetic substances may be toxic for human health. Since the pesticides are present on the surface of the berry, they will remain in solution after the crushing and fermentation because the wine is a hydroalcoholic and acid solution, thus highly solvent. The interaction of these substances with wine microorganisms may cause a considerable deterioration of the fermentation kinetics, increasing the risk of stopped fermentation and production of acetic acid and hydrogen sulfide. Considering the need to produce high quality and defect-free wines without any external contamination, we tested the technology of grape washing. The work shows the results of an extensive experiment designed to verify the effectiveness of grape washing on the reduction of pollutants, such as pesticides and metals, from the surface of the fruit and on the fermentation kinetics. The work was carried out on Corvina grapes with a washing and drying system provided by the manufacturer. The washing of the grapes reduced the quantity of polluting substances on the berries, thus obtaining a healthier product and improving the fermentative kinetics.

Funding Support: Perfect Wine Srl, Turatti Srl

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Diversity of Soil Microbial Communities is Influenced by Key Environmental Factors Due to Vineyard Land Use

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Soil microbial communities are important for maintaining crop production and ecosystem functioning. Agricultural land use can affect these communities, impairing their ability to benefit these production ecosystems. The goal of this study was to investigate how three soil microbial communities: bacteria, fungi, and arbuscular mycorrhizal (AM) fungi are affected by viticulture land use. We hypothesized that land use would influence soil microbial diversity and community structure, but that bacteria would respond more to differences in soil chemistry, while fungi would be affected more by differences in plant communities. Soil samples were collected from the plant communities found in 19 vineyard drive rows and 10 adjacent unmanaged sites throughout the Okanagan and Similkameen valleys, British Columbia, Canada. Biotic and abiotic factors were measured to characterize the individual sites and explain differences seen in the microbial communities. Using high throughput sequencing, we found that vineyard land use influenced both the diversity and composition of soil microbial communities. While AM fungi had higher species richness in unmanaged areas, bacterial richness was higher in vineyards. The community composition for all three microbial groups differed between the two land uses, with changes in bacterial communities correlating to soil pH, and changes in fungal communities (including AM fungi) correlating with changes in plant diversity. Other abiotic factors (cation exchange, temperature, and soil texture) were correlated with community compositional changes, but these were unrelated to vineyard land use. These results reveal key factors of viticulture land use that affect soil microbial communities, and could be used in developing a more natural, beneficial soil ecosystem in vineyard systems.

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

Influence of Novel Species of Grape Acetic Acid Bacteria on Yeast Fermentation

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Sluggish or stuck fermentations are a chronic problem in winemaking. One cause of stuck fermentation is a negative impact of bacteria arising from surface biota of the grapes present early in the fermentation. We isolated four different bacteria belonging to the genera *Acetobacter* and *Gluconobacter* from stuck

fermentation wine samples from three wineries. Previous research has shown that acetic acid bacteria can be directly inhibitory of yeast or induce the yeast [*GAR*$^+$] prion which is associated with a decrease in fermentative capacity, resulting in sluggish or stuck fermentations. Our research has shown that these four bacteria have a different sensitivity to SO_2. *Gluconobacter cerinus* was found to still grow at additional levels of 50 ppm of SO_2, while the other grape acetic acid bacteria were inhibited by this concentration under the same conditions. This preliminary result led us to hypothesize that the survival of these bacteria may cause a sluggish fermentation due to the induction of the [*GAR*$^+$] prion. To test the induction of the prion, fermentations were performed with EC1118 and UCD 932 yeast in co-cultivation with these different bacteria. Dialysis tubing created two relatively independent growing environments and was used to separate the yeasts and bacteria physically but not chemically. We performed the preliminary fermentation in a synthetic juice media, in order to provide yeasts and bacteria a relatively simple and controlled growth environment. The yeast isolated from the co-cultivation experiments will be screened for the [*GAR*$^+$] prion and compared to yeasts from control fermentations in the absence of bacteria. Fermentations are also being performed in grape juices to compare findings to those of the control medium. We aim to elucidate the proper SO_2 doses, which can suppress microbial contamination in the winemaking process, as well as the possible cause of stuck fermentations related to microbial contamination.

Funding Support: American Vineyard Foundation

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

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Rootstocks can be a valuable tool for viticulturists and confer numerous advantages, including vigor control. This research studied the F1 progeny from a Ramsey x Riparia Gloire de Montpellier cross, 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 hydraulic conductance, and that these variables can be associated with genetic markers. We evaluated 138 progeny from this population, three replicates each, for 60 days in a greenhouse. Each plant was pruned to a single shoot and watered daily. After day 45, 50 of the progeny were subjected to water deficit (50% of soil water content). Shoot growth rate, leaf area, and dry biomass were measured for the complete population, while hydraulic conductance, stomatal conductance, water potential, and chlorophyll were measured for the subset. The progeny showed transgressive segregation, and was clearly grouped, showing significant differences between small, intermediate, and big plants. Correlations between vigor vs. growth rate or biomass partitioning

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indices were highly significant (p = 0.00001). Shoot biomass vs. shoot growth rate showed a Pearson R value of = 0.77. Small plants had a higher roots/leaves ratio, but this was not observed for big plants. A PCA analysis also showed a strong role for shoot growth rate and biomass partitioning indices for vigor determination. Under water stress, larger plants showed lower specific hydraulic and stomatal conductances, indicating higher sensitivity to drought. Initial mapping efforts found one putative QTL explaining 10% of the variability for shoot growth rate on the consensus map of chromosome 17. QTLs related to growth have been found on chromosome 17 in other *Vitis* maps. QTLs with low but statistically significant effects can be important.

Funding Support: INTA EEA Mendoza, Argentina; California Grape Rootstock Improvement Commission; California Grapevine Rootstock Research Foundation; American Vineyard Foundation, CDFA Improvement Advisory Board, California Table Grape Commission, and Louis P. Martini Endowed Chair for Viticulture

Isolation and Purification of *Vitis vinifera* Grape and Wine Anthocyanins and Tannins

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Currently available methodology for the isolation and purification of grape and wine tannins and anthocyanins is both complicated and expensive, therefore hindering current research. A relatively simple and inexpensive method to separate these compounds using low-pressure chromatography has been developed. After initial purification on an XAD column, grape and wine samples (2013 Cabernet Sauvignon, Cold Creek vineyard) were separated on a silica gel column. Three distinct peaks were observed: first, a tannin peak; second, a peak containing malvidin-3-glucoside and its acetylated derivatives; and finally, a peak containing other minor anthocyanins found in *Vitis vinifera* grapes. The anthocyanins present in the second and third peaks can be further purified using preparative chromatography. However, grape and wine tannins are complex and cannot be easily separated using HPLC methods. An additional method has been developed to further separate and characterize the obtained tannin fraction. Using another silica column, the tannin peak can be separated into three distinct peaks based roughly on degree of polymerization. The first peak represents the largest procyanidin polymers, the second peak includes the smaller procyanidins, and the third peak contains the intermediate sized procyanidins. This new methodology enables both anthocyanins and tannins to be easily and inexpensively isolated, therefore opening a door to new research possibilities.

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

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Deep Subsurface Microirrigation for Increasing Vineyard Water Use Efficiency

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Research trials are underway to evaluate application of subsurface microirrigation without the use of buried lines. Water is distributed through standard suspended dripline via a pressure compensated emitter housed at the upper section of a PVC pipe that is placed vertically into the soil to depths ranging from one to three feet below the soil surface. The research hypothesis is that grapevine roots will extend deeper for access to this water source than those grown under surface drip systems to access the water. Our research objectives are to: 1) determine the capacity of vines to develop deeper and more robust root systems than typically occur under standard surface drip systems, 2) evaluate the water use efficiency of vines managed under each treatment, and 3) quantify the potential for more precise utilization of regulated deficit irrigation through subsurface microirrigation to maintain healthy vines and produce superior quality of grapes. We will compare physiological responses between vines irrigated with standard surface drip irrigation with those being watered with reduced amounts of water according to delivery depth, amount, and under continuous versus pulsed application schedules. Subtreatments also include length of irrigation period (full, half, and quarter) and pulsed versus continuous application to determine subsurface water distribution patterns under different soil types. Two of the three experimental locations are sited in commercial vineyards with cooperators. Battery powered controllers with latching solenoids are preprogrammed to schedule selected daily water amounts regardless of the actual irrigation period determined by the commercial vineyard irrigation managers and plots are located at the end of rows to minimize disruption of operations. Root systems are monitored by obtaining digital photographs within clear polyvinyl tubes (mini-rhizotrons) every two weeks.

Funding Support: WSU Emerging Research Initiative Northwest Center for Small Fruit Research Washington State Grape & Wine Research Program

Effects of Initial Sulfur Dioxide Addition on *Saccharomyces cerevisiae* Populations in Spontaneous Fermentations

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Sulfur dioxide is added to most commercially produced wines, prior to alcoholic fermentation as well as prior to bottling. It performs many functions for the winemaker, as it is both a strong antioxidant and an antimicrobial agent. The addition of sulfur dioxide prior to fermentation can help remove

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bacteria and yeasts that could spoil the fermentation. Although the effects of sulfur dioxide on spoilage bacteria and total yeast communities have been well documented, few studies have focused on the effects of this initial sulfur dioxide treatment on the *Saccharomyces cerevisiae* strains in alcoholic fermentation. This is important to determine because different strains of *S. cerevisiae* are known to produce different chemical and sensory profiles. This study aims to address this gap in knowledge by investigating the effects that three different levels of sulfur dioxide (0, 20, or 40 mg/L) have on the diversity and composition of *S. cerevisiae* strains spontaneously fermenting Pinot gris wine at a commercial winery. Each of these treatments was replicated in triplicate. The must was fermented in new 225-L French oak barrels until dry. Samples were taken for microbial analysis at the early, middle, and late stages of fermentation, as defined by sugar levels. Sulfur dioxide levels, both free and total, were also determined at each stage. The *S. cerevisiae* populations were analyzed and identified to the strain level by performing fragment analysis on microsatellite loci and comparing the resulting DNA fingerprint to that of published databases. Differences in strain diversity and composition between the different treatments will be presented and discussed. The results of this study will be of particular interest to winemakers who practice both inoculated and spontaneous fermentations, and who are looking to use fewer chemicals during winemaking.

Funding Support: Natural Sciences and Engineering Research Council of Canada

Impacts of Late Season Soil and Foliar Nitrogen Fertilizer Supplements on Winegrape Yeast Assimilable Nitrogen

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Nitrogen (N) application type and timing in winegrape (*Vitis vinifera* L.) production has been extensively studied in various grapegrowing regions and on countless cultivars. Due to the central role of N in vine growth and development, N status in the vineyard is carefully monitored and followed. Vines that are N deficient are provided with supplemental N to stimulate N metabolism and protein synthesis. However, vines that have access to surplus N respond with excessive vegetative growth, which can lead to a reduction in yield, reduced fruit quality, and/or a hospitable environment for pests and diseases due to increased canopy density. Since winegrapes in central Washington are historically low in yeast assimilable nitrogen (YAN), the objective of this study was to evaluate the impact of supplemental postveraison N applications of organic or conventionally sourced fertilizer, via soil and foliage, on irrigated Riesling winegrape. In 2011 and 2012, N applications were made by either soil (simulated drip) or foliarly at the rates of 0, 17, or 34 kg/ha N in a commercial vineyard. Fruit was harvested within 2 days of commercial harvest

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from each plot and quality measurements, YAN, and aromatic compounds were analyzed. Results from 2011 indicate that foliar N applications and organic soil applied N, at 34 kg/ha N rate, increased YAN, but this did not hold true for 2012. Many volatiles were detected when analyzed after each field season, and were found to differ, significantly at times. The differences in the volatiles between 2011 and 2012 are likely a reflection of the differences in the temperature and precipitation during the two growing seasons.

Funding Support: Northwest Center for Small Fruit Research, Washington Wine Grape Advisory Committee, Washington State University Agriculture Research Center

Impact of Different Cold Soak Durations on Cabernet Sauvignon Fermentation and Composition

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The phenolic content of red wine is responsible for many desirable sensory characteristics that directly affect wine quality, primarily color and mouthfeel. Winemakers have developed several enological practices aimed at maximizing or controlling phenolic extraction during winemaking. One such practice is cold soak, which is believed to increase both the extraction of anthocyanins and skin tannins. Cold soaks are periods of prefermentative maceration in which the must temperature is kept low enough to prevent spontaneous fermentation (8 to 15°C), with the length of the cold soak period varying from one to several days. Prior cold soak experiments performed by the authors showed that for Cabernet Sauvignon sourced from Lodi, CA, there were no significant differences in the extraction of anthocyanins and tannins as the length of the cold soak period increased, although skin tannin extraction decreased. In order to confirm our results from the 2013 harvest and to investigate the effects of increasing cold soak duration on a more phenolic rich fruit, triplicate research scale fermentations were performed using fruit sourced from Paso Robles, CA. Cold soak durations of zero, one, two, four, seven, and ten days were investigated. Daily fermentation samples were analyzed by UV-vis spectroscopy and HPLC to determine phenolic content. Finished wines will be analyzed six months after treatment to determine phenolic and oligosaccharide content. Preliminary results show increasing the cold soak duration had no effect on the rate of phenolic extraction. Minor differences in total extracted anthocyanins were observed, with anthocyanin concentration decreasing with longer cold soak durations.

Funding Support: E&J Gallo Wineries

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Applied Water Amounts and Rootstocks Interact on Productivity and Phenolic Composition of Zinfandel in Hot Climate

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Grapevine productivity and berry composition depend on interactions between the genotypes of the rootstock and the scion, cultural practices, and environmental factors. This study aimed to characterize the effect of rootstock genotype and applied water amounts on the productivity and phenolic composition of the grape berry in the hot climate. *Vitis vinifera* cv. Zinfandel grafted on either rootstock Freedom (high vigor, nematode resistant) or Salt Creek (high vigor, phylloxera and nematode resistant, and salt tolerant) was studied during two growing seasons under sustained deficit irrigation and regulated deficit irrigation. Water status, yield, and berry composition (sugars, gallates, flavonols, flavan-3-ols, anthocyanins, and total tannins) were measured at harvest during the two growing seasons. Applied water amounts affected berry mass, yield, water footprint, skin and seed mass, and concentration of anthocyanins but did not affect flavonols, flavan-3-ols, and tannins, regardless of rootstock. Rootstocks affected berry mass, yield, water footprint, skin and seed mass, concentration, and hydroxylation proportion of anthocyanins, in addition to flavonols and total tannins. Scion yield, water footprint, and berry composition differed greatly between the two rootstocks tested. Rootstocks and applied water amounts affected the concentration and hydroxylation proportion of anthocyanins, and Zinfandel was less sensitive to applied water amounts in the hot climate when grafted onto Freedom than when grafted onto Salt Creek.

Funding Support: American Vineyard Foundation

Analysis of Acetaldehyde Condensation Reaction Thermodynamics by 1D and 2D H-NMR

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The addition of oxygen to a red wine through barrel/bottle aging or micro-oxygenation is a beneficial step in the development of its color and aroma. Control over oxidation is vital for the use of oxygen to improve wine quality. As the main product of ethanol oxidation, acetaldehyde has been measured as a means of following wine oxidation. However, a number of wine substrates react reversibly with acetaldehyde and the equilibria that result complicate the analysis of this compound. Standard analysis methods likely do not reflect the amount of acetaldehyde produced through oxidation or even the concentration of free aldehyde present in wine at a specific time. As a means of

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quantifying free acetaldehyde without shifting these equilibria, 1D and 2D NMR methods have been previously investigated and applied to real and synthetic wine samples. This study implements these methods to measure the kinetic and equilibrium properties of acetaldehyde in a wine-like medium. Model wine solutions containing acetaldehyde and alcohols that form acetals were analyzed by ^{1}H NMR spectra and 2D homonuclear (^{1}H-^{1}H) correlation spectroscopy (COSY) with multiple solvent suppression to improve sensitivity. This data provided equilibria concentrations of the reactants and products, allowing for determination of equilibrium constants and, in some cases, the rate of formation could also be observed. This data, in addition to that for other acetaldehyde reaction products, can provide an accurate measure of the quantity of acetaldehyde present, as well as the amount produced by oxidation. The determination of free acetaldehyde in the presence of important wine substrates is ultimately necessary to assess and predict proper oxygen and SO_2 treatments.

Funding Support: American Viticulture Foundation

Influence of Fermentative Parameters on the Synthesis of Aromas by an Evolved Wine Strain of *Saccharomyces cerevisiae*

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Fermentative aromas play an important role in the organoleptic profile of young wines. Their production depends both on yeast strain and on fermentation conditions. To assess the combined effects of environmental parameters (temperature, initial assimilable nitrogen, and phytosterol contents) on the synthesis of these compounds, we studied their production by two *Saccharomyces cerevisiae* strains with different aromatic profiles: Lalvin EC1118 and Affinity ECA5, obtained by evolutionary engineering of Lalvin EC1118. First, by using a Box-Behnken design, we built a model describing the effects of the three environmental parameters and their interactions. The evolved and parental strains were strongly discriminated by their production of higher alcohols (except propanol) and acetate esters, which was systematically higher for the evolved strain regardless of the environmental conditions. These molecules were thus identified as metabolic markers of this yeast strain. On the contrary, the production of ethyl esters was not systematically different between the yeast strains. In a second step, we determined the kinetic profiles of fermentative aromas using an innovative online monitoring system of the concentrations of the main volatile compounds in the off gas during the alcoholic fermentation. The high measurement frequency (one per hour) makes the calculation of the rate and specific rate of production of these compounds

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possible, allowing a better understanding of yeast metabolism. We highlighted some differences in the chronology of production between the two yeast strains, suggesting some modifications in the regulation of aroma synthesis. In particular, a strain-dependent interaction between nitrogen and phytosterols was identified. This system also allowed a quantitative study through the calculation of production yields. The main difference between the parental and evolved yeast strains was the yield of conversion between a higher alcohol and its acetate ester, suggesting changes in this specific enzymatic activity resulting from the adaptive evolution strategy.

Funding Support: INRA (National Institute of Agronomic Research

Interspecific Hybrid Identification and Linkage Map Construction of a Chambourcin x Cabernet Sauvignon Population

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Vitis interspecific hybrid Chambourcin is a cultivar of largely unknown parentage which exhibits moderate cold hardiness characteristics and resistance to downy mildew. Chambourcin also displays resistance to grapevine vein clearing viruses (GVCV, personal communication Dr. Wenping Qiu). The purpose of this experiment is to generate an interspecific hybrid population by crossing *V.* interspecific hybrid Chambourcin and *V. vinifera* Cabernet Sauvignon. The ultimate goal of performing this cross is to create a cultivar with the disease resistance and cold hardiness of Chambourcin combined with the superior wine quality of *V. vinifera* Cabernet Sauvignon. The results from this experiment will also allow for the comparison of this population with a *V. aestivalis*-derived Norton and *V. vinifera* Cabernet Sauvignon population to analyze and compare disease resistance, cold hardiness, and berry quality results for the two populations. Simple sequence repeat (SSR) markers were used for identification of interspecific hybrids. Out of 215 plants tested, a total of 150 interspecific hybrids were identified (~70%). Following interspecific hybrid identification, ~800 SSR markers will be tested for polymorphism using the two parent samples and a set of six F_1 progenies. The informative polymorphic markers will then be run across the entire population. These results will be used to construct a linkage map.

Funding Support: Funding Support: USDA-NIFA NLGCA Capacity Building Grant 2013-70001-21268

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Impact of the Timing and Temperature of Malolactic Fermentation on Chardonnay Wine

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This study investigated the impact of malolactic fermentation (MLF) on Chardonnay wine, focusing on the timing and temperature of MLF. Chardonnay grapes from the Oregon State University vineyard were harvested, destemmed, and pressed. After settling overnight, juice was removed from the lees and 3-L aliquots dispensed into 4-L carboys. The commercial yeast product Prelude (*Torulaspora delbrueckii*) was added to one set of carboys and juice was held at 15 or 21°C for 48 hr. After 48 hr, the carboys were inoculated with *Saccharomyces cerevisiae* D47. Carboys of juice to which Prelude was not added were also inoculated with D47. At the time of D47 inoculation, half of the carboys were also inoculated with *Oenococcus oeni* Beta to induce MLF. For the remaining carboys, Beta was inoculated at the completion of alcoholic fermentation. Triplicate fermentations of treatments were performed at either 15 or 21°C. When D47 and Beta were inoculated simultaneously, MLF completed in less than 14 days at both temperatures. When Beta was inoculated after alcoholic fermentations, MLF took 28 days to complete at 21°C and 41 days to complete at 15°C. Acetic acid concentrations did not differ between treatments conducted at the same temperature. However, significantly higher acetic acid concentrations were present in wines fermented at 15°C compared to 21°C. Ethanol concentrations were significantly different between treatments performed at the same temperature as well as between wines fermented at 15°C compared to 21°C. The highest ethanol concentration was measured in wines fermented at 15°C where MLF occurred after alcoholic fermentation (14.6% v/v). The lowest ethanol concentration was present in wines fermented at 21°C where Prelude had been inoculated and MLF occurred simultaneously (13.8% v/v). Wines will be analyzed for volatile aroma compounds by GC-MS and assessed by a trained sensory panel.

Funding Support: Oregon Wine Research Institute

Comparative SSR Analysis of Some *Vitis sylvestris* (GMEL.) Accessions and *Vitis vinifera* L. Cultivars in Hungary

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The evolution of cultivated plants played an important role in the ascent of humanity. Research of their origin and evolution started at the beginning of

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the 20th century, but many questions still remain. There are many theories regarding the evolution of the European grapevine (*Vitis vinifera* L.). The *V. sylvestris* GMEL. in Hungary is a protected species, which is significant in terms of nature conservation and reserve of biodiversity as well. Based on theoretical and practical research, it is supposed that this species, or crossing it with other species, could be the progenitor of the European grapevine (*V. vinifera* L.). The ex situ conservation of the desired individuals has a great practical importance, as they can serve as a resistance source in future breeding programs. The microsatellite analysis of the grape can be traced back to the early 1990s, when many SSR loci were identified and characterized in grapes. In this study, the quest and the SSR analysis of the *V. sylvestris* GMEL. populations of the Szigetköz and FertőHanság National Park of Hungary are explored. Twenty different genotypes of woodland grape (*V. sylvestris* GMEL.), 10 cultivars of European grape (*V. vinifera* L.), and 10 species/genotypes of rootstocks were analyzed in 15 SSR loci of different linking groups. The preliminary results show that the analyzed *V. sylvestris* accessions form a distinct group, but are closer to the *V. vinifera* cultivars than to the rootstocks. This raises the probability that these woodland grapes are true-to-type *V. sylvestris*.

Funding Support: Hungarian Scientific Research Fund (project no. PD-109386)

Investigating the Role of Acetaldehyde in Tannin Modification and Color Stability of Red Wines

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Tannins play a major role in the sensory perception of wine, especially as it relates to astringency, and also contribute to the color stability of red wines by forming polymeric pigments from condensation reactions with anthocyanins. Both astringency softening and polymeric pigment formation are thought to be due to tannin oxidation reactions, which occur by direct reactions between tannins and anthocyanins, or through indirect, two-electron reactions involving carbonyl bridges. In this study, we aim to characterize the consumption of acetaldehyde in real and model wine systems due to reaction with wine tannins and anthocyanins. The extent and rate of acetaldehyde consumption was compared with analyses of the resulting tannins in model wine solutions. Samples were characterized for protein precipitation by tannins, total phenolics, polymeric pigment formation, tannin composition by phloroglucinolysis, and tannin content by MALDI-TOF MS. The reactions of acetaldehyde with tannins were also examined in a real wine (Cabernet franc) system. Tannins were characterized along with polymeric pigments to determine the degree of acetaldehyde modification. Results of this work suggest that acetaldehyde

concentration, wine pH, and sulfur dioxide concentration affect the rate of acetaldehyde consumption by wine tannins and anthocyanins. Additionally, acetaldehyde consumption was observed to correlate with the degree of tannin modification seen in real and model wines.

Funding Support: Graduate Competitive Grant Program, College of Agricultural Sciences, Pennsylvania State University; Pennsylvania Wine Marketing & Research Board, Pennsylvania Department of Agriculture

Grapevine Rootstocks: Root Development Variability and Responsiveness to Arbuscular Mycorrhizal Fungi

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Rootstocks play an important role in ensuring the quality and quantity of grapes that can be grown in different environmental soil conditions. Variations among different rootstocks reveal evolutionary differentiation, allowing growers to select rootstocks that will maximize grapevine vitality depending on the terroir they are being grown in. However, the role of fungal endophytes on the physiology of different rootstocks has not been investigated. This is important as grapevines are highly arbuscular mycorrhizal (AM), with AM fungi acting as an extension of a plant's root system, increasing its ability to absorb nutrients, tolerate abiotic stresses, and resist pathogens. These associations can significantly affect root traits such as branching intensity, average root diameter, and specific root length. The goal of this study was to determine whether rootstocks of different evolutionary background are affected differently by AM fungal colonization. In particular, we were interested in comparing how AM fungi affect the physiology and root system architecture of rootstocks. We predicted that mycorrhizal colonization would change rootstock architecture and that this would depend on the rootstock's evolutionary background. We compared eight different rootstocks of varying genotype and known growth characteristics. The vines were grown in a sterile medium, with and without mycorrhizae additions. Plants grew for 10 weeks in a greenhouse-controlled environment to allow mycorrhizal colonization to occur and influence vine physiology and growth. At harvest, root/shoot measurements were conducted, including root:shoot biomass ratio, root branching intensity, specific root length, average root diameter, and AM fungi colonization. The results will be discussed in the context of how interactions between rootstocks and AMF may be beneficial to establishing new vineyards.

Funding Support: Agriculture and Agri-food Canada (AAFC)

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Impact of Grape Maturity and Ethanol Concentration on the Composition of Washington State Merlot Wines

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Maturity is known to have significant impacts on the nonvolatile and volatile components in wine grapes, particularly during the exponential accumulation of soluble solids. Ethanol has numerous impacts on wine chemistry of sensory importance, including impacting the extraction of many nonvolatile compounds during maceration and the partitioning of volatile compounds between the vapor and liquid phase in finished wine. As there is a direct relationship between the resulting ethanol concentration and the initial soluble solids of a must, we sought to evaluate the interaction between ethanol and grape maturity during the crucial final stages of grape ripening. To this end, Merlot grapes from Washington State were harvested at three different maturities: ~20 Brix, ~24 Brix, and ~28 Brix. Chaptalization or saignée followed by water addition were used to target different ethanol concentrations at each harvest while maintaining the juice to skin ratios originally present in the must. Portions of each harvest were chaptalized or saignée followed by water addition to obtain nine treatments: three harvest dates at three soluble solids levels, which were fermented in triplicate. A trained descriptive panel (N = 14) rated the intensity of 16 aroma, 15 in-mouth flavor, 3 taste, and 3 mouthfeel attributes using a line scale. The panel differentiated the wines using attributes characteristic of green notes, including "herbaceous" and "vegetal" as well as attributes characterizing fruit notes, including "dark fruit," "red fruit," and "dried fruit." The volatile and nonvolatile composition of the wines was analyzed using a metabolomics approach (untargeted chemical analysis of biological systems). Multivariate analyses of peak heights from gas and liquid chromatography–mass spectrometry experiments showed that features detected by untargeted mass spectral analysis clearly differentiated the treatments in both the volatile and nonvolatile profiles.

Funding Support: Washington Wine Grape Funds, Fulbright New Zealand, New Zealand Institute for Plant & Food Research

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Genetic Study of Downy Mildew Resistance in *Vitis aestivalis*-derived Norton-based Population

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Downy mildew of grapevine, caused by the oomycete *Plasmopara viticola*, is one of the most widespread and destructive diseases of grapevine, particularly in viticultural areas with relatively high humidity. Traditional *Vitis vinifera* winegrape cultivars are susceptible to downy mildew, whereas several North American and a few Asian cultivars possess various levels of resistance to this disease. To identify genetic determinants of downy mildew resistance in *V. aestivalis*-derived Norton, a mapping population was developed in 2005 from a cross between *V. aestivalis*-derived Norton and *V. vinifera* Cabernet Sauvignon at the Missouri State Fruit Experiment Station, resulting in 95 hybrid progenies. This population was further expanded to 182 individuals by repeating the same crosses in 2011. A haploid Norton genetic map has been constructed with 373 simple sequence repeat (SSR) markers clustered in 19 linkage groups. In collaboration with VitisGen (www.vitisgen.org), a consensus map of 4,486 single nucleotide polymorphism (SNP) markers generated by genotyping by sequencing (GBS) has also been developed in this population. Disease progression and resistance reaction in response to *P. viticola* was studied eight days postinoculation in the given population using both visual and quantitative measures. Preliminary data from the quantitative trait loci analysis using both SSR and SNP markers will be compared, contrasted, and discussed.

Funding Support: USDA-NIFA NLGCA Capacity Building Grant 2012-70001-20164

Regional Differences between Typical Riesling Wines from Rheingau and Washington State

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The varietal aroma and taste of Riesling wines are highly receptive to growing conditions of the grape and wine production practices. The experiment was designed to compare commercial Riesling wines from Rheingau and Washington State. Olfactory and global sensory perceptions of regional typicality for 20 commercial Riesling wines (10 per region) from vintage 2012 were investigated in both regions by two panels consisting of wine experts with a high degree of specialization to Riesling. Chemical analysis of the wines focused on nonvola-

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tile compounds (organic acids and phenolics) and a selection of volatile aroma compounds (higher alcohols, fatty acids, esters, terpenes, norisoprenoids, and sulfur compounds). Additionally, different typicality concepts in the two regions were examined regarding the holistic and descriptive sensory space and chemical wine composition of the wines. Results show that Riesling wines could be differentiated according to their regional typicality. Moreover, it was shown that the wine experts from different regions did not necessarily share the same perceived sensory concept of typical Riesling wine. In order to clarify the concept of Riesling typicality from both regions, regional typicality was linked to particular sensory perceptions and to wine analytical compositional data. Significant regional differences were found both from chemical composition and sensory evaluation. Several sensory descriptors were linked to specific aroma compounds, such as varietal thiols, C_6-compounds and C_{13}-norisoprenoids, which explained the observed sensory difference between the regions. Our data suggest unraveling the links between sensory perceptions and wine composition data are essential for validating the differences between the regions and prominent compound and sensory relationships in Riesling wines. This allows further identification and optimization of viticultural and enological processes in order to obtain typical Riesling wines from different regions.

Funding Support: We would like to thank all the Washington and Rheingau wineries that donated the wines for the experiment

Effect of Crop Reduction of Vines Infected with Grapevine Red Blotch-Associated Virus on Fruit Maturity

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Grapevine red blotch disease has been highly prevalent in several California counties and other states. A DNA virus, Grapevine red blotch-associated virus (GRBaV), has been shown to be associated with diseased grapevines. The effect of red blotch disease was evaluated over two years in the same vines of Cabernet Sauvignon and Chardonnay cultivars located in Napa and Sonoma counties, respectively. Zinfandel vines with a history of large crop loads were also evaluated in Sonoma County in 2014. Vines were determined to be GRBaV positive or negative by qPCR assay as well as negative for all grapevine leafroll-associated viruses, vitiviruses, and nepoviruses. Total soluble solids (Brix) were significantly lower and malic acid greater in juice at harvest in all cultivars with the exception of Zinfandel ($p \leq 0.05$). Yield was reduced in 2014 in infected Chardonnay vines compared to vines that were not infected by GRBaV. Diseased grapevines had reduced cluster number, cluster size, and berries per cluster. Average berry weights of GRBaV-positive Chardonnay and Zinfandel vines tended to be greater while berry weights of Cabernet Sauvignon

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were not affected. Yields were not significantly different in Cabernet Sauvignon or Zinfandel, however lack of a significant difference in the latter may be due to greater variability in crop load. The effect of crop reduction was evaluated utilizing the same GRBaV infected Cabernet Sauvignon and Chardonnay vines as in 2013 to determine if there was a carryover effect. Flower clusters were removed to ensure fruit bearing shoots carried only single clusters. Total soluble solids, pH, and titratable acidity were not significantly different in juice samples from diseased vines regardless of total cluster number. These results indicate that dropping fruit to improve quality in vineyards with red blotch disease may not produce the desired effect.

Funding Support: American Vineyard Foundation

Proposal of the Head-Heart-Base System (HHB) as an Innovative Method to Describe and Evaluate the Flavor Profile of Wine

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The novel method proposed in this work combines advantages of several other sensory techniques for the evaluation of wine, while simplifying the tasting protocol, making it more accessible for unexperienced panelists. Another idea behind the new method is to reach significant differences between samples easier, thus making the sensory evaluation of similar wines more effective. While the differentiation between head, heart, and base aroma is already used in other areas like fragrance engineering, it is a novelty for the sensory analysis of wine. The head aroma is defined as the first impression after lifting a lid off the glass without stirring the sample. The heart aroma is defined as the main characteristic after swirling the sample. Finally, the base character is defined as the lasting aroma impression after intensive and retronasal evaluation of the wine. Every part is evaluated on its own using five groups of general descriptors (fruity, floral, spicy, ambient, and chemical) for basic profiling, while every impression can be refined further in a second step. To differentiate the samples not only based on a significant number of panelists agreeing on a group, the length of the triangle sides and the corresponding angles in a standardized graphic display can be used. The resulting value (HHB-value) reflects the complexity of the wine. Tests revealed that the technique is easy to learn and most panelists considered it easier to concentrate on aroma impressions stepwise following a strict tasting protocol. The general concept of a head, a heart, and a base note was well perceived and accepted because it makes it easier for judges to describe their time dependent aroma impression. Using a group like "fruity" instead of trying to decide which fruit it could be, also proved to be comforting for unexperienced judges and increased their motivation.

Funding Support: no external funding

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Quantifying Carbon Stocks in a California Vineyard

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Quantifying terrestrial carbon (C) stocks of vineyards represents an important piece for understanding C sequestration potential of these perennial cropping systems. Considering ~230,000 hectares in California are dedicated to wine-grape production, annual C capture and storage in wood may be considerable. In this study, grapevine C biomass was measured by destructive sampling of 72 individual 15-year-old Cabernet Sauvignon vines from a California Northern San Joaquin Valley vineyard. The objectives were to characterize C stocks in terms of allometric variation between biomass fractions of roots, wood, canes, leaves and fruit, then test correlations between easy to measure variables such as trunk diameter, pruning weights, and harvest index to vine biomass fractions. C stocks were also estimated from the volume of biomass in mounds generated during vineyard removal, and then compared with standing biomass values obtained previously. After 15 years of growth, our measurements of the perennial vine biomass yielded 9 Mg C ha^{-1} sequestered, whereas annual biomass (leaves and canes) was estimated at 1.7 Mg C ha^{-1}. The fruit produced contained 1.8 Mg C ha^{-1}. The diameter of the trunk was found to be highly correlated with the wood C stocks (r = 0.91) and the pruning weights were highly correlated with the leaf and fruit C stocks (r = 0.84). Carbon stock estimates in the mounds of vine wood (5.84 Mg C/ha) were not different than individually measured vines (5.90 Mg C/ha). This research demonstrates the potential to quantify vineyard C stocks with standing biomass measurements (e.g., trunk diameter) or mounded woody vine biomass at vineyard removal. Information collected from vineyard management practices could enable growers to record their current carbon stock and might also provide the basis to calculate future C stock estimations, especially important considering the significance that C sequestration is taking in the productive equation of agro-ecosystems.

Funding Support: The Nature Conservancy

Quantitative Method for Chiral Monoterpenes in White Wine by HP-SPME-MDGC-MS in a Different Wine Matrix

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Chiral monoterpenes are important characteristic compounds for a number of aromatic white wines and are responsible for floral and citrus aromas. How-

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ever, there is limited information on the identity and quantity of these chiral monoterpenes in white wine. This study developed a quantitative head space solid phase micro-extraction multidimensional GC-MS method using stable dilution isotope analysis for measurement of fifteen compounds; (S)-(-)-limonene, (R)-(+)-limonene, (+)-(2R,4S)-*cis*-rose oxide, (-)-(2S,4R)-*cis*-rose oxide, (-)-(2R,4R)-*trans*-rose oxide, (+)-(2 S,4S)-*cis*-rose oxide, furanoid (+)-*trans*-linalool oxide, furanoid (-)-*cis*-linalool oxide, furanoid (-)-*trans*-linalool oxide, furanoid (+)-*cis*-linalool oxide, (-)-linalool, (+)-linalool, (-)-α-terpineol, (+)-α-terpineol, and (R)-(+)-β-citronellol. Accuracy, limit of detection, limit of quantitation, reproducibility, and stability were all determined. A significant matrix effect was noted, resulting in a necessity for separate calibration curves for wines with different sugar content (dry, sweet). Injector and extraction temperature did not impact the adsorption of the compounds onto the SPME fiber or degradation of the compounds when injected into the GC. The limit of quantification ranges from 1 ng/L to 1.11 µg/L. The chiral monoterpene content of six Pinot gris wines and six Riesling wines were analyzed. ANOVA showed significant differences for all of the detected chiral compounds. Furanoid (2R, 5R)-(+)-*trans*-linalool oxide, furanoid (2R, 5S)-(-)-*cis*-linalool oxide, furanoid (2S, 5R)-(+)-*cis*-linalool oxide, (-)-α-terpineol, and (+)-α-terpineol were detected in all 12 white wines. (-)-Linalool and (+)-linalool were found in all six Riesling wines and three Pinot gris wines. PCA showed a clear separation between the Riesling and Pinot gris wines, with Riesling wines containing more chiral terpenes than Pinot gris wines. The Riesling wines were further separated into two groups on PC2. Further interest will be in determining which compounds and concentrations of these chiral terpenes play a role in sensory aspects of these wines.

Funding Support: Oregon Wine Board, Oregon Wine Research Institute

Establishment Technique Impacts the Morphology of Chambourcin Grapevines in Missouri

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An evaluation of establishment techniques for Chambourcin grapevines was conducted during the 2009-2012 seasons. Our objective was to determine if a more labor intensive establishment technique aimed at producing greater photosynthetic area during the establishment year would result in increased vine growth, precocity, and early fruit yield. The study was established in May 2009 as a factorial experiment comparing two root systems (own-rooted and grafted to 3309C rootstock) and four establishment methods (open-trained without protection-2 shoots, grow tube-2 shoots, milk carton-2 shoots, and fan-trained without protection-6 shoots). The fan-training method required more labor and complicated herbicide application. All vines in 4 of 12 field

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replications were destructively harvested in September 2009. Total leaf area was measured and vine tissues (above and below ground) separated for dry matter determination. The fan method significantly increased leaf area compared with the other methods, which were not different from each other. Dry matter for all above- and below-ground components except for the root shank (woody below ground tissue) was highest in fan trained vines. Root system treatment did not impact total leaf area or dry weight. However, grafted vines had increased trunk and root shank dry weights compared with own-rooted vines. Fruit yield and clusters/vine were not affected by establishment treatment in 2011 or 2012. However, grafted vines exhibited greater fruit yield and clusters/vine than own-rooted vines in 2012. While the additional labor invested in fan training increased leaf and total vine mass, it did not result in increased precocity or early yield at this site.

Funding Support: Missouri Wine and Grape Board Missouri Wine Marketing and Research Council University of Missouri Cooperative Extension Service

Population Dynamics of *Saccharomyces cerevisiae* Strains during Controlled Multistarter Fermentations at Different Nitrogen Levels

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Nitrogen is the most important growth limiting substrate during wine fermentation. Deficiency of nitrogen is one of the main causes of stuck and sluggish wine fermentations. Yeast strains have different requirements for nitrogen during wine fermentation. Although China is one of the fastest growing wine producing countries in the world, little has been done to characterize the competitiveness and nutritional requirements of indigenous Chinese yeast strains. To understand yeast interactions of indigenous Chinese strains with commonly used commercial yeasts, changes in yeast ratios of mixed inocula populations of *Saccharomyces cerevisiae* were analyzed over the course of fermentation in synthetic juice. Fermentations were performed in duplicate with five different inoculants: three pure yeast cultures and two initial 1:1 mixtures of strains at two nitrogen concentrations. Relative population ratios during fermentations were tracked with antibiotic resistance markers. The results showed an early death of *S. cerevisiae* UCD522 during the mixed fermentation with UCD2610 which was expected since strain UCD522 is sensitive to the killer strain UCD2610. The ratio of killer to sensitive cells was >10000:1 at day 2 and thereafter. In terms of multistarter fermentations with no killer activity, the Chinese strain NX11424 and UCD522 competed with each other throughout the timecourse of fermentation. There was an increase in the ratio of NX11424 to UCD522 of 10:1 at day 2 despite an initial inoculum ratio of 1:1. The ratio of NX11424 to UCD522 decreased midfermentation, then rose again at the end of the fermentation with high nitrogen. Population dynamics are also being evaluated at a

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low nitrogen level. In the future, metabolomic analysis will be used to elucidate metabolic interactions in multistarter fermentations. This study will contribute to the understanding of interactions of indigenous Chinese strains with commercial wine strains, leading to improvement of the wine quality in China.

Funding Support: China Agriculture Research System (CARS-30-jg-03), China Scholarship Council

Impact of Grapevine Red Blotch Disease in Red-Berried Winegrape Cultivars

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Grapevine red blotch (GRBD) is an emerging viral disease in many grapevine-growing regions in the United States. We have studied impacts of GRBD in own-rooted, red-berried winegrape (*Vitis vinifera*) cultivars (Merlot and Syrah) planted in two geographically separate commercial vineyards. For this purpose, we have identified 15 pairs of grapevines in Merlot and Syrah blocks in such a way that an individual pair has one symptomatic and one non-symptomatic grapevine next to each other in the same row. Selected pairs of vines were tested by PCR for the presence of Grapevine red blotch-associated virus to ensure that symptomatic and non-symptomatic vines are positive and negative, respectively, for the virus. Impacts of GRBD on total chlorophyll, chlorophyll *a* fluorescence and photosynthesis were measured in cvs. Merlot and Syrah during preveraison and postveraison stages. Overall results indicated that chlorophyll fluorescence and photosynthesis are unaffected in asymptomatic leaves of virus-infected vines during preveraison, but are significantly affected in symptomatic leaves of virus-infected vines when symptoms are apparent during postveraison. Weight of cane prunings per vine, which is a measure of growth during the preceding season, was less by 39.34% in GRBD-infected Merlot vines and 56.67% in GRBD-infected Syrah vines compared to their respective healthy vines. GRBD-infected Merlot vines produced fewer number of clusters per vine (a reduction of 20.39%) with a 25% reduction in total fruit yield per vine compared to healthy vines. Similarly, GRBD-infected Syrah vines produced fewer number of clusters per vine (a reduction of 31.10%) with a 51.6% reduction in total fruit yield per vine compared to healthy vines. The data indicate that GRBD can cause significant reduction in fruit yield and affect vine physiology in both Merlot and Syrah. However, the impact of GRBD appears to be variable between winegrape cultivars studied.

Funding Support: WSDA-Specialty Crop Block Grant Program (Project No. K1275), Wine Advisory Committee of the Washington Wine Commission, Agricultural Research Center, WSU-CAHNRS

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Yeast Assimilable Nitrogen Optimization for Cool-Climate Riesling: Fermentation Performance and Wine Composition Effects

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It is known that an adequate yeast assimilable nitrogen (YAN) concentration is necessary for successful wine fermentation, and supplementing musts with nitrogen is a common industry practice. In the Finger Lakes region of New York, Riesling is a signature grape variety which previous studies suggest often has YAN concentrations below the 140 mg/L considered a practical minimal limit. To test the influence of must nitrogen concentration on desirable sensory characteristics, we investigated the impact of YAN range on varietal expression in Riesling. In the fall of 2013, a Riesling must of 20 Brix and 120 mg/L initial YAN was supplemented with diammonium phosphate (DAP) to increase YAN to 180, 250, and 300 mg/L. Each supplemented fraction and the unaltered control were inoculated with three different *Saccharomyces cerevisiae* yeast strains: EC1118 (*Lallemand*), Epernay II (*Red Star*), and W15 (*Lallemand*). As expected, the results were highly dependent on the yeast strain used. EC1118 was able to ferment wines to dryness (<2.0 g/L residual sugar) in all cases, with fermentation rates increasing at higher YAN levels. The Epernay II control did not reach dryness, but all treatment wines fermented completely. Wines fermented with W15, classified by the vendor as high nitrogen requirement yeast, all finished with residual sugar around 5 g/L, and fermentation rates did not increase with DAP supplementation. Consumption of YAN was also monitored, and residual levels after fermentation completion ranged from 5 mg/L (W15, all treatments) to 105 mg/L (Epernay II, 300 mg/L initial YAN). Those results suggest that excessive DAP supplementation may promote slight improvements in fermentation rates, but may also result in high levels of nitrogenous compounds postfermentation, posing a potential microbiological risk. Current work is focused on determining the aromatic composition of the finished wines and assessing any detectable sensory differences among treatments.

Funding Support: Federal Formula Funds (FFF) - NYSAES

Use of NGS in Conjunction with PMA to Determine the Relative Abundance of Viable Yeast and Bacteria Species in Wine

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Culture based methods, used to monitor microbe communities in wine fermentations, are more labor intensive, relatively inaccurate, and produce relatively low numbers of sequences, as compared with next generation

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techniques. Illumina Miseq next-generation-amplicon-sequencing (NGS) can generate up to 20 million sequences per run, which can result in the calculation of relative abundance for both bacteria and yeast species in wine samples. NGS has been used to determine relative abundance of species, but it hasn't been used in a way that differentiates between viable and dead cells of wine samples. Propidium monoazide (PMA) is a photo-reactive DNA binding dye, which through photolysis, causes permanent DNA modification of dead cells and renders them unamplifiable by polymerase chain reaction (PCR). Previous research has used qPCR to successfully determine the number of viable wine yeasts in samples that had been treated with PMA. The primer sets, BITS&B58S3 (amplifies the ITS1 region of yeasts) and F515/R806 (amplifies the V4 domain of 16S rDNA bacteria), used in a previously conducted next generation study, show high accuracy in predicting taxonomic classification of total wine microbes, but not necessarily of those that are living. Our study provides evidence that the combination of PMA and NGS can enable us to determine the relative abundance of viable wine bacteria and yeast without any interference from dead cells. NGS preparation utilizes the same fundamental technique of PCR. PMA treatment was shown to dramatically reduce dead cells from being detected. We also showed that reduced PCR cycles during NGS preparation reduced amplification bias. Future wine research, which utilizes the above techniques, can potentially provide an accurate way to monitor living microbial communities in wine fermentations.

Funding Support: NSERC, BC Wine Grape Council (BCWGC), Quail's Gate Winery

Foliar Application of Yeast Derivatives on Grape Quality and Resulting Wines

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New applications for yeast derivatives in the vineyard are intended to improve grape quality. Some have proven to be useful to increase the production of plant secondary metabolites which are clearly involved in the quality of the grape for wine production. In this study, the foliar application of substances based on 100% yeast derivatives RD-LM and RD-LA on cv. Cabernet Sauvignon and cv. Sauvignon blanc, respectively, were studied. The trends in changes due to these products to agronomic parameters, berry composition, and wine quality were examined. These yeast derivatives specifically designed to be used with the patent foliar application technology WO/2014/024039 were applied at veraison in a commercial vineyard in Albacete, Spain. Results of the experiments across maturity stages showed differences between treatments in two of the three main grape berry parts in both varieties studied, with

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a higher percentage of skin and lower percentage of flesh in treated grapes than in nontreated grapes. These differences seem to be related with the increased levels of total and extractable anthocyanins and tannins found in the treated grapes. No differences in plant physiology, vegetative growth, yield components, total soluble solids, pH, or total acidity were shown between treatments. Treatments with yeast derivatives were associated with consistent effects on mouthfeel and positive aroma attributes in the sensory analysis conducted. The effect of RD-LM and RD-LA indicated that such foliar application treatments could be regarded as useful for improving phenolic composition and wine quality, supplementing or replacing common viticulture practices.

Funding Support: Universidad Politécnica de Madrid

Time of Flowering and Seed Content Contribute to Variable Entry of Pinot noir Fruits into the Ripening Phase

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For *Vitis vinifera*, ripening is considered asynchronous because fruits within a cluster variably enter the ripening phase. Why this occurs is unknown, although asynchronous flowering has been suggested as a source. Seeds may have an effect as well because hormones derived from the seed influence fruit development. The purpose of this study was to determine the contribution of flowering time and seed content on fruit entry into the ripening phase (veraison). A noninvasive technique was used to tag flowers on their day of anthesis and relate flowering time to how far resulting berries progressed into the ripening phase at veraison. The seed content of each berry at veraison was also measured. The collective contribution of flowering time and seed content to ripening progress was estimated using a linear, mixed effect model. Berries' advancement in the ripening phase were well defined by the proportion of their weight occupied by seed(s) (SB), with high SB berries typically starting to ripen later than low SB berries. Developmentally lagging and advanced berries were often the result of late and early flowering events, respectively. However, differences in developmental duration between flowering and veraison were insufficient to explain the diversity of ripening classes from each flowering day and how fruits with equal SB but from different flowering days were seemingly equivalent at veraison. This study examined an ecologically interesting behavior in a culturally and economically important crop, documenting probable contributors to ripening asynchrony.

Funding Support: Oregon State University

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Investigation of a Novel Polymer-Based Fining System for the Removal of 2-Aminoacetophenone from *Vitis vinifera* Wines

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The biogenic amine 2-aminoacetophenone (2AAP) contributes to aroma profiles that are described as "foxy" or "native." This aroma is characteristic of *Vitis labrusca* and *V. rotundifolia* grapes, which are popularly grown in the northeastern United States. The foxy/native aroma of 2AAP is not associated with wines made from *V. vinifera* grapes, and is thus considered to be a defect in *V. vinifera* wines. The presence of 2AAP in *V. vinifera* wines has been attributed to endogenous formation via atypical aging as well as inadvertent addition through odorant scalping by winery equipment used to produce *V. labrusca* wines. We hypothesized that 2AAP could be selectively removed from tainted wine with a novel polymer-based fining system that takes advantage of the diffusion properties of low molecular weight aroma compounds such as 2AAP. This design features cation exchange resins encapsulated within a polydimethylsiloxane casing to create conditions that would support continuous diffusion of 2AAP into the capsule. Results from our laboratory confirm the removal of 2AAP from model wine by this method. The selectivity of these capsules to remove 2AAP only was evaluated by GC-MS by comparing the overall aromatic profile of commercially produced white wines spiked with 2AAP before and after treatment. These findings provide the foundation for the development of an innovative method for the removal of 2AAP from finished *V. vinifera* wines.

Funding Support: Pennsylvania Wine Marketing and Research Board

Can Grapevine Diseases be Managed by Cover Crop-Mediated Changes in Soil Microbial Diversity?

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Two economically important diseases in vineyards are young vine decline and damage from the lepidopteron pest, climbing cutworm. A solution to both of these problems could lie in vineyard floor and soil management. There is mounting evidence that diverse plant communities and their associated microbes are major drivers of plant health and productivity. Establishing a cover crop that maximizes this phenomenon in vineyard soil may influence vine health by affecting diversity of mutualistic AM fungi, the abundance of plant pathogens, and the abundance of fungal entomopathogens that affect the vine indirectly by decreasing herbivore populations like cutworm. We conducted a

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greenhouse pot experiment to measure the effect of cover crop vegetation on grapevine health through plant induced changes in the soil microbial community. We used a feedback approach to determine how different cover crop mixes influenced both growth and disease incidence on a grapevine planted in soil trained under different cover crop vegetation. Treatments included nonnative orchard grass, native grass mix, native grass mix plus flowering native plants, native grass mix plus legumes, resident vegetation from a 20+ year fallow field, and tillage. Soil was collected from replicated experimental plots established in 2011 at the Pacific Agri-food Research Center in Summerland, B.C., and dormant rootstocks were planted to each trained soil and grown for three months. We evaluated each treatment based on vine growth, AM fungal colonization, root lesions, and survival of cutworm larvae. In this presentation, we will discuss how growers can incorporate soil microbial diversity into their management plans.

Funding Support: AAFC (Agriculture and Agri-food Canada)

Characterization of Mexican Wine by Headspace Solid-Phase Microextraction-Gas Chromatography and UV-visible Spectroscopy

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Mexican wines have become internationally accepted for their quality; however, little or no information has been published about their physicochemical characteristics. Forty-six monovarietal wines (Cabernet Sauvingnon, Malbec, Merlot, Tempranillo, Nebbiolo, and Syrah) from different Mexican wine regions: Aguascalientes, Baja California, Coahuila, Queretaro, Zacatecas, and the emerging Chihuahua region were studied. Samples have been analyzed by headspace solid-phase microextraction-gas chromatography-mass spectroscopy, identifying 19 volatile compounds, the most abundant of which were alcohols and esters. Wines also have been tested to determine total polyphenols, total anthocyanins, total acidity, and pH. Wines made in Chihuahua were the richest in total anthocyanin content. Folin index of samples was in the range of 20–50. Only 18 samples among the 46 were within the ideal pH range (3.3–3.6), with the rest above this parameter. Total acidity in more than half of the samples was below 4.5 g/L, the minimum established value for alcoholic beverages in Mexico. High pH and low acidity are expected in Mexican wines due to the high temperatures in the areas where wine regions are located.

Funding Support: Fundación Produce Chihuahua A.C.

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Trace Metal Content in Mexican Wine

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Analysis of trace metals in wine is important not only because of quality concerns, but also for the potential impact on consumer health. It is known that trace metals in wine come mainly from the soil where grapes are grown (including pesticides, fungicides, and fertilizers) and from equipment used during wine production. Trace metals were quantified in 24 monovarietal commercial red wines from different Mexican regions (Aguascalientes, Baja California, Coahuila, Queretaro, and Zacatecas). Wines were analyzed for Al, Cd, Cu, Fe, Hg, Mn, and Pb using atomic absorption spectroscopy. Average results were: Al, 1.16 mg/L; Cd, 0.01 mg/L; Cu, 0.66 mg/L; Fe, 1.54 mg/L; Hg, 4.91 mg/L; Mn, 1.51 mg/L; Pb, 0.43 mg/L. Some of the samples showed high levels of Pb, Hg, and Cu. No correlation was found among grape variety, wine region, or winery for samples of high content of trace metals.

Funding Support: Centro de Investigación para los Recursos Naturales

Handler Dermal and Inhalation Exposure to Sulfur Dust Applications on Grapevines

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Dermal and inhalation exposure estimates are essential for risk assessments of pesticides. Risk assessors lack adequate exposure data for agricultural handlers of pesticide dust applications. We used whole body dosimeters and face/neck wipes to estimate potential dermal exposure (PDE) and personal air sampling pumps to estimate potential inhalation exposure (PIE) of handlers while they loaded and applied sulfur dust. This study describes how potential exposures depend on application equipment (tractor cab and duster models) and work activities. We monitored handlers performing ground applications in six California counties. Fourteen handlers pulled a duster with an open cab and 11 drove an enclosed cab tractor. All handlers loaded and applied a product containing 98% sulfur dust as the active ingredient (AI). The medians for total pounds AI handled were significantly different between open and enclosed cab ($p < 0.005$ using Wilcoxon rank sum test with continuity correction). There-

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fore, we normalized PDE and PIE by total pounds of AI handled to adjust for the differences in amount of sulfur dust handled. Both PDE and PIE remained significantly higher in handlers in open cabs compared to the enclosed cab even after normalization ($p < 0.001$ for both PDE and PIE). PIE had a smaller contribution to potential total exposure compared to PDE. Handlers spent more than half of the workday performing high exposure activities (applying and loading), with a greater amount of time spent applying relative to loading. The remainder involved low exposure activities (break/standby, handling PPE, and other activities with no observable sulfur dust exposure). The potential dermal and inhalation exposure was highly variable, therefore additional studies are needed to determine the effect of duster make and model, meteorological conditions, and canopy density on PDE and PIE of handlers to sulfur dust.

Funding Support: Department of Pesticide Regulation

Metabolomic Assessment of the [*GAR*+] Prion State with UCD932 in Model and Chardonnay Juices

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The robustness of *Saccharomyces cerevisiae* fermentations is indispensable for the winemaking process. However, as we have previously shown, the fermentative capacity of *S. cerevisiae* can be reduced by the presence of spoilage organisms capable of inducing the [*GAR*$^+$] prion in yeast. Yeast harboring [*GAR*$^+$], a heritable genetic element, display slowed fermentation kinetics, reduced competitiveness, and a range of other physiological changes. Surprisingly, the transcriptome of [*GAR*$^+$] by microarray showed relatively few changes in expression, except for a 40-fold down regulation of ubiquitous sugar transporter *HXT3*. Due to this, we hypothesize significant metabolic changes will help explain observed phenotypes of [*GAR*$^+$]. With the goal of understanding global changes occurring within yeast harboring the [*GAR*$^+$] prion, we performed a metabolomics screen. Samples were pulled for analysis from fermentations carried out minimal must media (MMM) and UC Davis (UCD) Chardonnay with UCD932 in the prion negative [*gar*$^-$] and prion positive [*GAR*$^+$] state. We have previously observed differences in the production of volatile compounds from [*gar*$^-$] vs. [*GAR*$^+$] in MMM and juice. This combined with other observed physiological differences led us to hypothesize that other metabolic changes are also occurring inside [*GAR*$^+$] cells. We expect to see differences in central carbon metabolism, stress response pathways, lipid metabolism, and metabolites related to regulation of cellular homeostasis. Our fermentations were performed with UCD932, genetic variants of UCD932, and Lalvin EC-1118. A first set of fermentations in MMM, a defined synthetic

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media, allowed us to establish a baseline for the metabolite pool produced by [*gar*$^-$] and [*GAR*$^+$] yeasts without the complicating matrix effects of grape juice. We then screened [*gar*$^-$] vs. [*GAR*$^+$] in UCD Chardonnay to assess how the effects of the prion affected yeast metabolism in applied enological processes. We aim to elucidate biomarkers and provide insight into the metabolic differences underlying [*GAR*$^+$].

Funding Support: American Vineyard Foundation

Investigation into the Genetic Basis of Leaf Shape in Grapes

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Leaf shape is a distinguishing taxonomic characteristic in grape (*Vitis* spp.) that displays great diversity. Beyond being a reliable indicator for species and cultivar identification, leaves also provide information about the function of the plant as it adapts to its environment. However, little is known about the genetic basis of leaf shape anatomy in grapes. In this project, phenotypic analysis using morphometrics and genotypic analysis using single nucleotide polymorphism (SNP) markers have been combined to better define the genetic basis of grape leaf shape. Our mapping population consists of the parents, *V. aestivalis* Norton and *V. vinifera* Cabernet Sauvignon, and their 182 F1 progeny, located at the Missouri State University Fruit Experiment Station. For phenotyping, we have identified important leaf shape characteristics (n = 17) based on the venation pattern, lobes, and sinuses of each leaf. Morphometric analysis of phenotypes will be completed to quantify aspect ratio (AR) and venation patterning. In collaboration with VitisGen (www.vitisgen.org), approximately 43,320 SNP markers generated by genotyping by sequencing (GBS) have been identified in this population. Statistical analysis will be performed using a general procrustes analysis (GPA) to produce trait measurements in the form of principal component (PC) scores. Correlation analysis using both genotyping and phenotyping data will be calculated to identify quantitative trait loci (QTLs) responsible for leaf shape.

Funding Support: Missouri State University Faculty Research Grant

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Impact of Environmental Factors on Peppery Aroma in *Vitis vinifera* L. cv. Shiraz Wine. A Modeling Approach

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Rotundone is a sesquiterpene that gives grapes and wine a desirable peppery aroma. Previous research has suggested that growing grapevines in a cool climate is an important factor that drives rotundone accumulation in grape berries. This study used historical data sets to investigate which weather parameters are mostly influencing rotundone concentration in grape berries. Furthermore, historical weather data was compared to the rotundone concentration of wines made in corresponding seasons. Models were constructed based on the Gompertz function to describe the evolution of rotundone concentration in berries in the ripening process. This characterization is an important step to potential prediction models to assess the final quality of the resultant wines based on specific compounds behavior with the environment. For this purpose, wines produced from 15 vintages from the same Shiraz vineyard (The Old Block, Mount Langi Ghiran, Victoria, Australia) were analyzed for rotundone concentration and compared to comprehensive weather data obtained by interpolated information available from the vineyard site using the piecewise cubic hermite interpolating polynomial method (PCHIP). Results showed that the highest concentrations of rotundone consistently were found in wines from cool and wet seasons. The principal component analysis (PCA) showed that the concentration of rotundone in wine was negatively correlated with daily solar exposure and grape bunch zone temperature (veraison to harvest) and positively correlated with vineyard water balance. The modeling techniques described in this paper were able to estimate the behavior of rotundone concentration based on seasonal weather conditions and grapevine phenological stages, and could be used to predict the final rotundone concentration early in future growing seasons. This could enable the adoption of precision irrigation and canopy management strategies to achieve favorable, or mitigating adverse impacts, of microclimatic variability within a vineyard, with the aim of maximizing the rotundone concentration of grape berries.

Funding Support: Australia Grape and Wine Authority, University of Melbourne

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Impact of Severe Leaf Removal on Methoxypyrazine Content and Vine Function of Merlot

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Methoxypyrazines are volatile compounds that have an aroma described as bell pepper and are linked to grape ripening. Low fruit zone temperatures and inner canopy light levels are known to result in higher concentrations of methoxypyrazines in winegrapes. Cultural practices such as basal leaf removal can be used to improve canopy microclimate in order to reduce methoxypyrazine content and improve wine quality. This project investigated the effects of severe primary shoot defoliation on vine balance and fruit quality of Merlot. Specific treatments investigated the combination of both decreased vegetative growth and higher canopy light and temperature level results on methoxypyrazine content of Merlot grapes. Three levels of leaf removal treatments were applied to the morning side of the canopy one week after fruit set during the 2014 growing season in Southern Oregon. Weekly measurements included canopy light interception and temperature, enhanced point quadrat analysis, vine shoot growth, leaf gas exchange, and stem water potential. Fruit and wine composition analysis at harvest included soluble solids, titratable acids, pH, total anthocyanins, and polyphenolics. Methoxypyrazine content in berries was determined using MDGC-MS. Measurements showed that severe leaf removal significantly decreased the number of canopy leaf layers and fruit zone light interception and increased berry skin temperature. Severely defoliated vines also slightly improved vine balance as indicated by lower Ravaz index values compared to nondefoliated vines. Sensory analysis of the wines indicated elevated green aromas in treatments with less severe leaf removal, with corresponding high levels of methoxypyrazines. The information gathered in this project will benefit growers in adapting primary shoot defoliation to achieve more balanced vines, reducing methoxypyrazines, and achieving desired wine quality.

Funding Support: Oregon Wine Research Institute

Managing Astringency and Stickiness of Grapevine and Wine Tannins of Merlot Grapevine in the Hot Climate

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An experiment was conducted in the San Joaquin Valley of California on Merlot to determine the interaction of mechanical leaf removal (control, prebloom, post-fruit set) and applied water amounts [sustained deficit irrigation (SDI) at (0.8) and regulated deficit irrigation (RDI) at 0.8 (budbreak-fruit set) – 0.5

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(fruit set-veraison) – 0.8 (veraison-leaf fall) of estimated vineyard evapotranspiration (ET_c) on how to manage flavonols, the astringency of berry skin tannins, and measure the stickiness of tannins in wine. Both quercetin and myricetin were greater with either prebloom and post-set leaf removal application, while applied water amounts did not affect either. Post-fruit set leaf removal increased epicatechin-(4β-2)-phloroglucinol extension unit, catechin terminal subunit, and total skin tannins. Irrigation did not have any effect on any subunits measured. The mean degree of polymerization was slightly reduced with the application of leaf removal treatments, while irrigation did not affect it. The tannins in wine were slightly greater with the leaf removal treatments and the stickiness of wine tannins was greater with the SDI treatment. This study provides fundamental information to growers on how best to manage astringency and stickiness of tannins in the hot climate without adversely affecting yield.

Funding Support: American Vineyard Foundation ARI

Leaf Removal and Deficit Irrigation Effect on Grape Texture and Phenolic Composition

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It is generally accepted in the wine industry that grape physical properties are related to grape quality and wine composition. It is also known that structural and chemical properties of the skin cell walls determine mechanical resistance and the ability to release phenolic compounds during processing. The effect of different viticulture practices on grape composition and texture were investigated. Three leaf removal and two deficit irrigation treatments were applied in a vineyard located in California and planted with Merlot. Grape texture and phenolic composition were determined at commercial harvest and wines were produced from the same lots. Results revealed correlations between berry texture and grape total anthocyanin content. It has been determined that skin elasticity is negatively correlated to an increasing total concentration of anthocyanins in grape skins. Regulated deficit irrigation increased grape skin elasticity and lead to higher concentration of malvidin-3-glucoside. Prebloom and postset leaf removal treatments reduced berry hardness and elasticity and enhanced anthocyanin production in skins, which reflects the importance of light exposure in both biosynthesis of grape anthocyanins and degradation of skin tissue.

Funding Support: American Vineyard Foundation, Bronco Wine Company

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Susceptibility of Different *Muscadinia rotundifolia* Cultivars to GFLV

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Grapevine fanleaf degeneration caused by grapevine fanleaf virus (GFLV) is one of the most serious diseases facing grapegrowers worldwide. The virus is transmitted by *Xiphinema index*, the dagger nematode. *Muscadinia rotundifolia*, a valuable source of resistance in grapevine breeding programs, is known to be highly resistant to *X. index* but its immunity to GFLV is not clear. The objective of this work was to characterize the GFLV susceptibility of different cultivars of *M. rotundifolia*, and VR (*vinifera* x *rotundifolia*) and VM (*Vitis* x *Muscadinia*) hybrids. *Muscadinia* plants were approach grafted to GFLV-infected Chardonnay under greenhouse conditions. Newly grafted test plants were cut away from the scion donor two months after grafting and assayed for GFLV, using RT-qPCR, four months after grafting. First results showed that GFLV was detected in Trayshed, although levels were 10 times lower than susceptible St. George. We will present results obtained with cultivars Lucida, Trayshed, Pride, Southern Home, Southland, Topsail, Dixieland, and VR hybrid O39-16.

Funding Support: California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, American Vineyard Foundation, CDFA Improvement Advisory Board, California Table Grape Commission, and Louis P. Martini Endowed Chair for Viticulture

Grip22c, a Multi-Functional Gene from Chinese Wild Grapes *Vitis quinquangularis*

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Grapes are among the widest cultivated fruit crops in the world. Our previous study showed that genotypes from *Vitis quinquangularis* had significantly higher resveratrol contents than *V. vinifera* grapevine cvs., especially the cv. Danfeng-2, which contains much higher levels of resveratrol than all other *V. quinquangularis* genotypes in berry development stage. However, its molecular regulations are still not fully known. The aim of this study was to investigate this molecular regulation in grape berry proteome during ripening in *V. quinquangularis* cv. Danfeng-2 through the Isobaric tags for relative and absolute quantification (iTRAQ). iTRAQ results showed that Grip22 was highly differentially expressed across four time points in Danfeng-2 berry. The use of annotation, analysis tools, and literature findings led to obtaining the protein

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(Grip22) and track functional genes (Grip22a, Grip22b, and Grip22c), which were predicted functional in the berry development stage. Further semiquantitative real-time PCR analysis validated that the protein Grip22 expression resulted from the gene Grip22c. This gene was isolated, characterized, and transformed into tomato (cv. Micro-Tom) and grape (cv. Thompson Seedless) to provide more information about its potential functions during ripening. Results showed that the higher expression of protein Grip22 in *V. quinquangularis* was found in accordance with the increased resveratrol accumulation in grape during berry development, and this accumulation was found also with the higher expression of Grip22c in the transgenic lines. Further, Grip22 was observed positively correlated to the pathogen resistance in studied transgenic plants. Findings of this study will improve the established cultivars with admirable horticultural traits and will also offer more incentive to explore this novel gene and Chinese wild grapes.

Funding Support: National Science Foundation of China (Grant No. 31372039)

Pinot noir Wine Aroma Composition as a Result of Changes in Vine Balance

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Canopy management and crop thinning are widely used practices in winegrape production worldwide, as they influence crop load (canopy size relative to fruit yield) and impact wine quality. However, optimized crop load guidelines have not been defined for Oregon Pinot noir. The influence of vine balance on Pinot noir wine composition was investigated in a three-year trial in Oregon's north Willamette Valley. Vines were managed with two vineyard floor management practices to allow various vine vigor levels, and two crop thinning levels were applied to vines. Wines were produced at harvest and evaluated for their volatile profiles. Forty compounds including both the free- and bound-forms of C_{13}-norisoprenoids were quantified. Principle component analysis results indicated that yield level did not influence wine aroma profiles, but different levels of vine vigor led to very different wine volatile profiles in 2011 and 2012. However, there was no impact of vine vigor on the wine volatile profile in 2013. The 2011 and 2012 vintage wines had more branch-chain esters and some branch-chain alcohols in wines from lower vigor vines compared to higher vigor vines.

Funding Support: Oregon Wine Board

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Carotenoid and C_{13}-Norisoprenoid Composition of Pinot noir Grapes from Vines of Differing Vine Balance

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Grape-derived aroma compounds are important for variety specific aroma of wines. Previous studies have shown that C_{13}-norisoprenoids such as β-damascenone and β-ionone are important grape-derived compounds for nonfloral grape varieties, as they contribute berry and stone fruit aromas in wine. The C_{13}-norisprenoids are derived from carotenoid degradation during berry development. Carotenoid breakdown and C_{13}-norisoprenoids synthesis in Pinot noir grapes were investigated in a vine balance study during 2013. Fruit samples were collected across six dates from veraison to harvest within a viticulture experiment designed with two crop thinning levels (full crop and moderate thinning) applied to vines with two different vine vigor levels (tillage and grass cover). Results indicated that the vine vigor level had a greater influence than crop thinning level on berry carotenoid composition. Similarly, vigor level influenced the C_{13}-norisoprenoids in grape berries more than crop thinning level. Grape berries from high vigor vines consistently had higher carotenoids and free form C_{13}-norisoprenoids than the grapes from lower vigor vines. However, for the hydrolytically released C_{13}-norisoprenoids, the difference between high vigor and low vigor were not different. The multivariate analysis also showed some interactions between sampling time, vine vigor, and yield factors on the composition of carotenoids and C_{13}-norisoprenoids.

Funding Support: Oregon Wine Board

Analysis of Volatile Phenols in Wine by EG/PDMS-based Stir Bar Sorptive Extraction and GC/MS

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Volatile phenols typically contribute off-flavor in wine and their concentrations need to be reliably monitored. Volatile phenols in wine are typically analyzed by HPLC. However, the HPLC method has a high detection limit and chromatographic interference. In this study, an ethylene glycol (EG)/polydimethylsiloxane (PDMS) copolymer-based stir bar sorptive extraction (SBSE)-GC-MS method was developed for the analysis of volatile phenols (4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, and 4-vinylguaiacol) in wine. The wine samples were diluted with phosphate buffer (1 M, pH = 7) and extracted with an EG/PDMS stir bar. Volatile phenols were thermally desorbed and analyzed by GC-MS. Parameters affecting extraction efficiency including ionic strength, pH, extraction time, ethanol content, and nonvolatile matrix

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were studied. Good correlation coefficients with R^2 in the range of 0.994 to 0.999 were obtained for volatile phenol concentrations of 5 to 500 µg/L. Recovery for all phenols were 81.4 to 97.6% in a wine matrix. The method had a standard deviation less than 5.8% for all volatile phenols. The limit of quantification was lower than 3µg/L, which was significantly lower than the values in previously published papers using either SPME extraction or PDMS stir bar sorptive extraction. The method was successfully applied to analyze different types of wines, including red and white wines.

Funding Support: Self-funded

Effect of Juice YAN Composition on Formation of Volatile Compounds in a Model System

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The effect of nitrogen concentration and composition on formation of volatile compounds was investigated using a synthetic grape juice system. Synthetic grape juices with varying yeast assimilable nitrogen (YAN) concentrations and compositions were inoculated with either *Saccharomyces cerevisiae* strain UCD522 or strain P1Y2. Nitrogen concentration and composition of the juice was manipulated by altering the amino acid and ammonia content of the juices. Low YAN juice contained a total of 112 mg/L YAN where 81 mg/L YAN was derived from primary amino acids and 31 mg/L was derived from ammonia (added as diammonium phosphate (DAP)). The YAN juice contained 346 mg/L YAN where primary amino acids provided 315 mg/L while ammonia again provided 31 mg/L YAN. A high DAP juice was prepared where YAN content was 350 mg/L, but the majority of the YAN was provided by ammonia (269 mg/L). Aside from nitrogen composition and concentration, all other components were identical between the three synthetic juices. Fermentations were performed in triplicate at 21°C. At the completion of alcoholic fermentation, the wines were assessed for fermentation esters, alcohols, and fatty acids by SPME-GC-MS. The results showed that higher YAN concentration produced higher levels of free fatty acids and ethyl esters. However, when the high YAN content was provided primarily by ammonia, lower free fatty acids and corresponding ethyl esters were produced.

Funding Support: Oregon Wine Board

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Industry

Innovative Tools to Keep Spoilage Microbes Out of the Wines

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Microbial contamination and its consequences are major threats to wine quality and are often undetected until spoilage is noticeable by sensory evaluation. Capable of developing under difficult conditions, at any time during a wine's life, spoilage microbes are opportunist organisms and are very difficult to eliminate. They are responsible for chemical and physical changes in the wine. In order to prevent and control the development of spoilage microorganisms, the winemaker must be proactive with an early detection of microbes in wine and cellars. Maintaining good cellar hygiene, controlling alcoholic and malolactic fermentations, and protecting wine during aging are essential in managing microbe populations. Recent developments have made new tools available for the winemaker to remove the microbes that are responsible for wine spoilage through fining, thereby avoiding filtration. Chitosan is a natural polysaccharide, produced by de-acetylation of chitin, extracted from *Aspergillus niger*. Chitosan interacts with microorganisms via charge attraction and degradation of cell walls. Activated forms of chitosan have been shown to be effective in removing several groups of spoilage microrganisms. This work summarizes several trials done with wineries in various winemaking countries and illustrates the effect of activated forms of chitosan on the most notorious and feared spoilage microbes, such as *Brettanomyces*, *Lactobacillus*, *Pediococcus*, *Oenococcus*, or even *Acetobacter*. Preliminary results indicate that a fining with activated chitosan is able to eliminate high contamination levels of these spoilage microbes, making it a promising new tool for microbial control in the winery.

Funding Support: Enartis USA Inc, dba Enartis Vinquiry

Color Stability: Impact of Tannins, Polysaccharides, and Use of Microoxygenation

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Color is the first quality observed in wine and thus one of the most important characteristics to consider when assessing the quality of a wine's organoleptic properties. The initial color of red wine is mainly due to free anthocyanins which are extracted from the grapes. Free anthocyanins will precipitate if not stabilized. The formation of stable pigment can occur via pigmentation with polyphenols or co-condensation. Copigmentation is an association of anthocyanins with a copigment such as flavonoids, catechins, mannoproteins, tannins, anthocyanins, or gums. Copigmentation is promoted by the presence

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of tannins and polysaccharides. Condensed anthocyanins, formed via direct or covalent bonds between anthocyanins and tannins, are the most stable colored molecules. The presence of tannins and oxygen encourages their formation. Several trials have been done in collaboration with wineries to evaluate the impact of tannin and polysaccharide additions during alcoholic fermentation and the use of microoxygenation between alcoholic and malolactic fermentation on color stability. We choose to work on red hybrid grape varieties, characterized by producing wines rich in anthocyanins but unstable, due to their low tannin content. The different forms of polyphenols are analyzed in the grapes, after both fermentations and after four to six months of aging. Microoxygenation has a strong impact on the ratio of free anthocyanin and pigmented tannins, which favors the color stability. Tannin and polysaccharide additions during alcoholic fermentation promote the increase of pigmented tannins while the free anthocyanins are decreasing, thus the color is more stable.

Funding Support: Enartis USA Inc, dba Enartis Vinquiry

Monitoring Soil Water Tension to Manage Irrigation and Grapevine Stress—from Wireless Sensors to Managers' Browsers

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A 2013 research project was designed to answer three questions: 1. Can soil water tension readings be returned to a vineyard manager's browser in real-time to help guide irrigation and canopy management decisions? 2. Does soil water tension, measured wirelessly with Watermark sensors, have a high correlation to grapevine water tension, measured by pressure chamber? 3. Does soil water tension equal or better soil volumetric water as a proxy for managing grapevine stem water stress? To answer these questions, 18 wireless sensor stations were installed across six vineyards in Oregon's Willamette Valley, with Watermark soil tension sensors installed at 1', 2', and 3' depths, beside a Sentek capacitance sensor access tube, near a drip emitter. Measurements of soil water tension, air temperature, and humidity were reported wirelessly at 15 minute intervals from sensor station to cellular gateway, to online database to manager's browser. Measurements of soil volumetric water (with a Sentek Diviner 2000 capacitance probe), midday stem water potential (with a PMS pressure chamber), and grapevine transpiration (with a Decagon leaf porometer) were made at weekly intervals. The answer to the three initial questions was "Yes," "Yes," and "Yes." Soil water tension in the grapevine root zone had a >90% correlation with grapevine stem water tension, which was equal or better than the correlation with soil volumetric water. This high correlation between soil water tension and stem water tension was found in low, medium, and high stress Pinot noir vine-

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yards, with end of season stem water tensions of 7, 10, and 13 bars, respectively. In conclusion, soil water tension reported wirelessly from sensor stations in real time can be used by vineyard managers to make irrigation decisions and manage grapevine stress, through the season and from year to year.

Funding Support: NW Vineyards Consulting

Development and Characterization of a Yeast Protein Extract as an Alternative to Wine Exogenous Fining Agents

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Modern enology must adapt to new demands on wine essence preservation and consumer safety. Consequently, current usage of fining agents derived from chemicals, vegetables, or animals, notably those containing allergenic substances, is already regulated (regulation (EU) 579/2012) and will be particularly controlled in the future. As slight fining efficiency of different yeast protein fractions have been shown by former studies, this work first focused on characterizing active flocculating parts of these products in comparison with the usual protein fining agents. A clear correlation between oligomer molecules (>15kDa) and fining efficiency on red wines has been confirmed. Based on these results, a specific industrial production process has been developed in order to extract, concentrate, and preserve high molecular weight native proteins from yeast cytoplasm, while keeping the resulting yeast derivative totally soluble. About 30% of molecules contained in this yeast protein extract were above 15kDa, of which more than 60% were oligomer, excluding sugars. In wine application, the yeast protein extract showed similar clarification properties as the widely used gelatin and egg albumen at the same dosage with thinner and compact lees. In addition, its preventing action on wine oxidation sensitivity was proven, and the organoleptic profile of the treated wines showed enhanced aromatic expression and mouthfeel by removing bitter components and harsh tannins. This yeast protein extract is totally soluble with immediate dissolution properties due to its microgranulated form. Exclusively derived from yeast, it is completely integrated in wine elaboration and as such represents an advantageous alternative to other wine exogenous fining agents.

Funding Support: Fermentis – a Lesaffre Division

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Development and Study of a New Decanter Technology on Grape Juice Extraction and Clarification

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The use of a centrifuge separator with horizontal axe (Decanter) for the continuous extraction of must from grape in a single solution can stand as a technological proposal, surely interesting for the processing of grape from mechanical harvest. This technology, though already tested in the past, has had no actual diffusion yet, mostly due to some criticality of the process. In particular, musts separated from solid parts of the grape tend to be characterized by high turbidity. After a series of experiences during the 2013 and 2014 harvest, a new technology has been used. This includes using a decanter to prepare a clear must for fermentation and to overcome the limits of must turbidity. The process has been patented and is based on the use of apt enzymatic compounds and clarifiers directly on the grape mash before using the Decanter. Our study will focus on: decanter performances during cellar applications using a clarifying agent in order to produce a clear must; characterization of solid parts in the must obtained with the decanter, compared with must obtained from pneumatic press; monitoring the oxygen content dissolved in the must, and evaluation of the effect of inert gas application; and microbiological differences of the grape juice obtained by traditional process (pneumatic press and flotation) and by the innovative process with the decanter technology. This new technology is suitable for grape harvested mechanically with no use of crusher-stemmer, presses, lines for must handling, decanting systems, floating, and filtration. A short process (both in terms of time and number of steps) is therefore achieved in order to enhance the quality of wine and, at the same time, reduce the production costs and the use of resources (water, power, etc.), thus improving the sustainability of the winemaking process from white grape.

Funding Support: Perfect Wine srl, Alfa Laval spa

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Effect of Carboxymethyl Cellulose on Tartrate Salt, Protein, and Color Stability of Red Wine

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Recent studies have confirmed a long-term effect of carboxymethyl cellulose (CMC) for tartrate salt stabilization in white wine. It has been argued that CMC is not only less effective in red wine but also interacts with proteins and polyphenols, generating turbidity and change in color. To explain these effects, we studied in detail the impact of CMC on haze formation and color stability of red wine. The influence of CMC concentration was tested with ten samples of red wine produced from several grape cultivars. The haze-forming material was analyzed by sodium dodecyl-sulfate polyacrylamide electrophoresis and the protein composition by high-performance liquid chromatography-mass spectrometry. Color alteration was documented by Vis-spectroscopy. Three samples of wine that were tested developed significant turbidity when treated with enological doses of CMC. The haze formation coincided with a high color density and protein instability of the wine. The insoluble fraction contained pathogenesis-related or late vintage wine proteins (thaumatin-like proteins, lipid transfer protein) and was associated with coloring matter. Carboxymethyl cellulose is of value for tartrate salt stabilization in red wine. Occasionally, it promotes development of protein haze and color loss. Initially, this behavior appears to limit the enological suitability of CMC, but it might also be considered as a new tool to remove unstable wine proteins. The results indicate the conditions under which red wine turbidity is triggered by CMC. Thus, they are important for the elaboration of recommendations for the optimal use of the polymer.

Funding Support: Erbsloeh Geisenheim AG

Decoloration of Wine and Subsequent Enzymatic Quantification of Histamine

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Histamine can be produced in wine as a result of bacterial decarboxylation of histidine. While low concentrations of histamine are involved in local immune responses as well as regulating physiological processes, uptake of high concentrations of histamine can cause toxicological effects. Since different recommendations exist for wine in the world, there is a permanent need for analysis with a simple and robust sample preparation method and determination with high precision and a good recovery. Current methods for analysis of hista-

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mine are HPLC, ELISA, and fluorimetry. We present a simple decoloration procedure for (red) wine and the subsequent enzymatic method for the specific quantification of histamine. The test kit for sample preparation contains three solutions that are used to decolorate wine by two simple precipitation steps within 15 min. The enzymatic test kit consists of a microtiter plate coated with two substrates and ready to use reagents (buffer, calibrators, and enzyme histamine dehydrogenase). The determination is performed within 15 min and the measurement wavelength is 450 nm, which is the common wavelength for ELISA procedures. The linear range is from 1 to 20 mg/L in the extracted sample (corresponding to 2.4 to 48 mg/L in wine) and the recovery is >85% in red wines and more than 90% in white and rosé wines. The limit of quantification is calculated to 1 mg/L. Wines at this low histamine levels showed CVs of less than 7%. The assay shows a high precision with a relative interlaboratory reproducibility standard deviation of less than 7%. The procedure contains no dilution steps, no washing steps such as for an ELISA, and a clearly defined end point of the enzymatic reaction. The procedure is flexible and scalable: it permits a high throughput capacity and also allows to running only a few number of samples as well.

Funding Support: R-Biopharm AG

Comparison of Traditional Versus Sap Flow-Based Irrigation across Multiple Sites and Multiple Years: Impact on Water Saving

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By monitoring vine water use continuously, new irrigation strategies can be implemented and compared with traditional irrigation. To evaluate the benefits of a new irrigation approach, we combined an experiment with vineyards across multiple geographic locations with an experiment across multiple years. First, we implemented a sap flow sensor-based approach to irrigation under contrasted locations and climates throughout California. Second, by continuously monitoring vine water use, we triggered irrigation based on real time vine water deficit and compared with traditional irrigation. Third, we investigated the temporal stability of the result by testing the carryover effects of sap flow-based irrigation measuring various fruit parameters. Last, we wanted to demonstrate that more conservative irrigation strategies could be adopted, even in a context of drought, while evaluating the consequences of irrigation reduction on yield and sugar loading. Six vineyard sites were selected and evaluated in a split treatment trial to compare the effect of two irrigation strategies in Sonoma, Napa, and Paso Robles in 2014. At one location, the treatment was

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implemented for three consecutive years. Under traditional strategy, irrigation was based on empirical knowledge, visual observations, and climatic forecasting. Under experimental strategy, we computed a daily vine water deficit index combining sap flow sensor data with climatic data. Irrigations are triggered each time the vine water deficit index reaches a threshold value. Results show that irrigation could be reduced by 40 to 100% in the experimental treatments even in a context of drought. No yield losses were reported and sugar accumulation was observed at a similar rate across treatments. By designing a framework that incorporates plant sensing data from a selection of reference sites, we can extrapolate irrigation decisions under contrasted soils and climates and over multiple seasons. Our study concludes that plant sensing irrigation promotes more conservative irrigation and improves vineyard economics.

Funding Support: Metropolitan Water District of Southern California, Bureau of Reclamation, Southern Nevada Water Authority, and Central Arizona Project. Fruitions sciences SAS Private wineries

New SBSE Techniques for Aroma Chemicals in Beer and Wine Offer Wider Recovery Range of Analyte Polarity

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More than 800 volatiles including alcohols, esters, aldehydes, ketones, volatile acids, terpenes, and pyrazines contribute to wine aroma. Selecting an extraction technique prior to GC-MS analysis has been challenging for such a wide array of analyte types with concentrations in the mg/L to a few ng/L range. While stir bar sorptive extraction (SBSE) has been shown in the past to offer the analytical advantages of automation, the possibility of using very small sample sizes, and solventless extraction, it suffered from low recovery with respect to polar analytes. As SBSE has matured, techniques have been developed to dramatically increase recovery of these important flavor and aroma compounds. This paper will show how the application of sequential SBSE, multi-SBSE, and the new ethyleneglycol-modified Silicone-coated (EG-Sil) stir bar now offer more uniform enrichment of aroma and flavor compounds in beer and wine.

Funding Support: None

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

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Color and Tartaric Stability on "Early to Market" Red Wines

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Traditionally, winemakers in the United States did not consider the treatment of red wines to achieve color and tartrate stability prior to bottling. This was primarily due to the conditions of the aging process naturally stabilizing the wine over time, and secondarily because the wine consumer didn't perceive these sediments as a serious defect. In the context of "early to market" red wines, the importance of reaching colloidal stability has become crucial. Because of their short aging period, red wines do not have time to reach equilibrium and become stable. On the other hand, a large portion of the color in young red wines originates from semi-stable pigments which are made of weak bonds that can break easily, leading to the loss of color and the production of deposits in the bottle. Protective colloids such as CMC, arabic gums, and mannoproteins have been widely used in the wine industry for the stabilization of potassium bitartrate salts on white and rosé wines; however, their use in red wines still remains limited in the U.S. For red wines, color stability brings an extra layer to the colloidal matrix, creating greater interactions with the stabilizing agents. The goal of this study was to evaluate the effect of four distinct treatments and compare their efficacy on the colloidal stabilization of "early to market" red wines. Preliminary results indicate that each stabilizing colloid treatment has an optimal range of action. The best results were found on treatments that combined CMC with arabic gums. In addition, mannoproteins proved to be a good solution for medium to low instabilities, in particular for higher end. These results provide a range of application that can be used as a guideline for winemakers to make appropriate decisions based on the status of their wines.

Funding Support: Enartis USA

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