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**65th ASEV
National
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TECHNICAL ABSTRACTS



AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

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Enology — Microbiology Session

Fermentation Performance of Four *Saccharomyces cerevisiae* Yeast Strains in Difficult to Ferment Chardonnay Juice

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Despite efforts to optimize the conditions of fermentation, some wineries still encounter fruit that is perennially difficult to ferment. This study focuses on a Chardonnay with a history of long, sluggish fermentations. The goal of this research was to screen a large collection of yeasts to identify strains that are capable of efficient fermentation of this juice. Of 14 strains screened, 12 completed fermentation in 7 to 9 days. Cell viability was lower in difficult juice for 10 strains. Of the remaining strains, three showed little to no difference in viability and one had greater viability in difficult juice. Two native isolates and one sequenced strain were chosen from among these for further evaluation. UCD2118, -2176, and -2780 were evaluated in six replicate trials for information on fermentation kinetics and viability, and metabolomic analysis was conducted at day 4. Strain UCD2778, observed to have reduced cell viability in difficult juice, served as a sequenced control. Strain UCD2778 fermented to dryness in both juices, but fermentation rate in the difficult juice exceeded the easy juice at 72 hr, and cumulative weight loss was greater in difficult juice thereafter. Difficult juice viability was not significantly lower. Like the control, strain UCD2780 had a higher fermentation rate in difficult juice at 72 hr. Strains UCD2176 and UCD2780 also exhibited greater cumulative weight loss in the difficult juice. Strain UCD2176 fermentations stuck at 8 to 9 Brix, with easy juice fermented further than difficult by about one degree. For strains UCD2780 and UCD2176, cell viability dropped more quickly in difficult juice than in easy juice during fermentation. Strain UCD2118 showed no juice-based difference in either cumulative weight loss or cell viability. Results of metabolomic analysis are pending.

Funding Support: American Vineyard Foundation

Interactions between *Brettanomyces bruxellensis* and *Oenococcus oeni*: Impact on Growth and Hydroxycinnamic Acid Reduction

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This study investigated interactions between *Brettanomyces bruxellensis* and wine lactic acid bacteria (LAB) and the impact on growth and volatile phenol production. Previous work had demonstrated that while *B. bruxellensis* could only utilize free hydroxycinnamic acids, one *Oenococcus oeni* strain evaluated could degrade tartaric acid-bound hydroxycinnamic acids. Continued assess-

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Enology — Microbiology Session — CONTINUED

ment of wine LAB and *B. bruxellensis* strains resulted in no further organisms demonstrating the ability to degrade tartaric-bound hydroxycinnamic acids. Additionally, studies investigated the impact of *O. oeni* on *Brettanomyces* growth and volatile phenol production. Studies in acidic grape juice (AGJ) broth (pH 3.50) demonstrated that growth of *O. oeni* could inhibit *Brettanomyces* growth in a strain-dependent manner. For example, while *B. bruxellensis* reached a maximum population of 4×10^7 cfu/mL in 7 days in the control treatment, *B. bruxellensis* populations only reached a maximum population of 6.7×10^6 after 10 days growth in broth where *O. oeni* strain CSO1 had previously grown. When *B. bruxellensis* was inoculated into Pinot noir wine where *O. oeni* strain CSO4 had performed malolactic fermentation (MLF), populations quickly decreased below detectable levels and did not recover during the experiment (50 days). This decrease in population was further explored by inoculating *B. bruxellensis* into wine either immediately following completion of MLF (three *O. oeni* strains evaluated), six weeks postcompletion of MLF, or after sterile filtration of wine following MLF. *B. bruxellensis* inoculated into sterile-filtered wines exhibited little to no growth inhibition compared to the control, while inoculation into unfiltered wines caused a delay of growth and lower populations than the control. Additional experiments will determine whether the observed growth inhibition of *B. bruxellensis* is due to the presence of live or dead *O. oeni* cells.

Funding Support: The Northwest Center for Small Fruits Research and the Oregon Wine Board

Tracking Microbial Regionality from Vineyard to Winery through Early Stages of Postharvest Fruit Processing

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The microbial consortia of wine fermentations can be significantly impacted by microbial contributions from both grape-surface microbes in the vineyard and from processing equipment and surfaces in the winery. Differences among microbial communities within grapegrowing regions and among varieties have been observed and likely contribute to a unique microbial regionality of wines, especially in the early stages of grape processing and fermentations. To investigate how grape-surface microbial communities are preserved or altered during harvest and pressing/crushing, five grape varieties from six different blocks in two separate vineyards were sampled in the weeks leading up to harvest. Two methods for sampling grape surfaces were used: swabbing whole clusters and aseptic berry collection. After harvest, must and juice samples from the corresponding vineyards were collected at various points during pressing and crushing. The yeast and bacterial communities of all samples were profiled

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Enology — Microbiology Session — CONTINUED

using culture-independent tools based on high-throughput next-generation sequencing. The variability of microbial profiles within vineyards, among varieties, and through grape processing was examined. Results demonstrate complex patterns of microbial establishment across vineyards, varieties, and grape processing steps.

Funding Support: Adolf L. & Richie C. Heck Memorial Fellowship, Horace O. Lanza Scholarship, Mario Tribuno Memorial Fellowship, Wine Spectator Scholarship (for MLS)

Identification of Wine-related Microorganisms by MALDI-TOF Mass Spectrometry

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Matrix-assisted laser desorptive/ionization time of flight (MALDI-TOF) mass spectrometry has increasingly been used to identify medically important microbes over the last five years. For 10 to 30 years, taxonomists have used ribosomal DNA sequencing to distinguish species of yeast and bacteria. In the MALDI technique, total proteins are extracted and a mass spectral fingerprint is created that is characteristic for a specific species. Ribosomal proteins are a large fraction of the proteins from logarithmically growing cells, so the taxonomy is directly correlated to the current taxonomy. The technique has the advantage of being rapid and inexpensive. However, MALDI must be adapted for use in fields other than medicine. The commercial databases have been designed for medically important organisms; therefore, new libraries of spectra must be created for the microbes found in other environments of interest. We have used the Bruker MALDI Biotyper to create databases for acetic and lactic acid bacteria commonly found in wine. We demonstrate that these databases are extremely well correlated with the rDNA sequence identification. While the technique has been successful for distinguishing species, there have also been attempts to extend the technique to look at strain differences as well as species differences. At the strain level, the protein extraction is very sensitive to the growth phase and the number of cells used. Varying the conditions has led us to 70 to 80% accuracy in strain typing for *Saccharomyces cerevisiae*. Strain analysis of *Brettanomyces bruxellensis* is continuing.

Funding Support: University of California, Davis, Department of Viticulture and Enology, Shields Endowed Chair in Dairy Food Science

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Viticulture — Vine Water Stress Session

Correlation between Soil Moisture, Sap Flow, and Subsequent Growth and Development in Merlot

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Is prebudbreak sap flow (bleeding) in grapevines directly associated with the ability of buds to break and with subsequent growth? To answer this question, a trial was conducted with pot-grown Merlot using loamy sand and sandy loam soils. Soil moisture was maintained at 1% (v/v) intervals between field capacity and permanent wilting point (PWP). At PWP, vines could neither bleed nor break bud. Vines grown slightly above PWP had delayed and very limited bleeding followed by nonuniform budbreak; subsequent shoot growth was stunted, many inflorescences were aborted, and the remaining inflorescences experienced poor fruit set. Bleeding rates and shoot growth were strongly correlated with soil moisture in both soil types. Shoot vigor (elongation and leaf expansion rates) was greatest when soil moisture was close to field capacity. Preliminary tests showed that if vines were initially maintained at PWP but watered back to field capacity (before they died), then they could resume normal budbreak and shoot development. Results suggest that soil moisture during budbreak is more important than previously thought. Also, soil moisture within about 3% (v/v) of field capacity maximizes early growth and yield capacity.

Funding Support: USDA-Northwest Center for Small Fruit Research, Washington State University-Wine Advisory Council, Altria Group, Inc., and Ste. Michelle Wine Estates

Heat Waves and Water Deficit: Effects on Photosynthesis, Transpiration, and Chlorophyll Fluorescence of Malbec Grapevine

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Water deficit and heat stress are important threats to sustainable agriculture worldwide. Each event independently can severely affect plant growth and yield. When they occur together, their effect on the plant may have severe consequences or may be devastating. To evaluate possible interactions between heat waves and water deficit on key plant physiological processes, a greenhouse experiment was designed with two-year-old potted grown vines (*Vitis vinifera* L. cv. Malbec) at INTA Mendoza Experimental Station. During one week, vines were exposed to four treatments that resulted from the combination of two ambient temperatures (30°C and 45°C) and two water conditions (100% and 50% soil water content). We also evaluated the response of those key physiological plant processes to rewatering and reducing ambient temperature

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Viticulture — Vine Water Stress Session — CONTINUED

of the water-stressed and heated vines. Before, during, and after treatment, combinations were applied, stomatal conductance, photosynthesis, transpiration, chlorophyll content, and fluorescence were measured and fluorescence parameters were calculated. Interactions were found between temperature and soil water content for photosynthesis, stomatal conductance, transpiration, and shoot growth. Conversely, no interactions were found for chlorophyll fluorescence, PSII, and Fv/Fm. Vines under high temperature and low soil water content showed the lowest values for photosynthesis, stomatal conductance, transpiration, and shoot growth, and their reduction was dependent upon soil water content. Chlorophyll content and PSII were reduced by high temperature and soil water content independently. Fv/Fm was reduced by high temperature, but no effect of soil water content was observed. Noticeably, after the vines were rewatered and ambient temperature reduced, the values of these key physiological processes recovered the following day and no differences among treatments were recorded. This may indicate that nonpermanent damage occurred to the leaf photosynthetic machinery.

Funding Support: INTA

Changes in Berry Transpiration and Xylem Backflow during Grape Berry Development

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Understanding grape berry water relations is fundamental for directing vineyard irrigation management to enhance grape production and quality. Among the components of berry water relations, the pathways that water exits from the berries, i.e., berry transpiration and xylem backflow, are less well understood. Three *Vitis* cultivars (Concord, Merlot, and Syrah) were used to investigate the developmental changes in berry transpiration and xylem backflow. Berry transpiration rate fluctuated diurnally with vapor pressure deficit. Transpiration rate per berry was correlated with berry surface area. Transpiration rate increased initially from ~6 Brix (berries were green and hard) to ~13 Brix (berries were red/purple) and then declined with further ripening. Although this developmental trend was consistent among three cultivars, there was distinct varietal difference in the absolute values of the transpiration rate. Using a mathematical model of berry growth, developmental changes in vascular water flows (i.e., xylem backflow and phloem inflow) were quantified, based on the measured rates of berry transpiration, berry growth, and solute accumulation and sucrose concentration in the phloem sap of the berry pedicels. It was demonstrated that to fulfill berry sugar accumulation, the amount of phloem inflow was in excess of the berry growth requirement. The excess was mostly disposed of as xylem backflow, due to the low rates of berry transpiration. If no

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Viticulture — Vine Water Stress Session — CONTINUED

xylem backflow occurred, hypothetically, sucrose concentration of the pedicel phloem sap would have to increase during ripening and create large differences in concentration within the same cluster. Therefore, this scenario is highly unlikely, and it is proposed that xylem backflow is essential for disposing of excess water during berry ripening.

Funding Support: Department of Horticulture at Washington State University, Chateau Ste. Michelle Distinguished Professorship, Rhone Rangers

Irrigation Scheduling in Northeastern North America

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Irrigation scheduling in arid regions is straightforward and involves daily calculations of water needs based on evapotranspiration (ET), crop coefficients (K_c), vine area, and effective soil water storage capacity (S). The S factor is calculated from available water storage capacity, rooting depth, and an availability coefficient. Normally, water is applied daily or on alternate days for periods of 1 to 2 hours, but frequency and volume vary widely depending upon region, phenological stage, and whether mild water stress is desirable. In eastern North America, this pattern is uncommon; it is likely that water is applied weekly and only in exceptionally dry growing seasons. The reasons are threefold: (1) soils are typically fine-textured and consequently have higher % available water than most California and Washington vineyard soils; (2) lower ET values minimize water losses from vineyard ecosystems; and (3) frequency and volume of precipitation reduce, or often preclude, application of irrigation. ET values of 6 mm and K_c values of 0.75 create demand for 11.3 L/v/day (79.1 L/v/week) based on 100% ET replacement, whereas deficits of 12.5% ET require 1.4 L/v/day (9.9 L/v/week). Assuming 12 mm rainfall is subtracted from calculations as runoff, merely 14.4 mm rain over a weekly period eliminates the need for a 12.5 ET deficit application, while 31.4 mm is sufficient to preclude 100% ET replacement. Despite these conditions, irrigation has been effective in several vintages in the Niagara Region in Ontario (2001, 2002, 2004, 2005, 2007, 2010, 2012) in several winegrape cultivars (Baco noir, Chardonnay, Cabernet Sauvignon, Sauvignon blanc), as well as in juice grapes (Concord, Niagara) and table grapes (Sovereign Coronation) in terms of improving yield, berry composition, and wine quality. Positive responses to partial root-zone drying were also observed for Cabernet Sauvignon and Sauvignon blanc despite occasional rainfall and the likelihood of horizontal water movement in the soil.

Funding Support: NSERC, Grape Growers of Ontario, and Wine Council of Ontario

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Enology — Sensory/Chemistry Session

Correlating Wine Quality Ratings to Chemical, Volatile, and Elemental Profiles of Cabernet Sauvignons from California

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Twenty-seven Cabernet Sauvignon wines from nine California wine regions were selected for this study based on their performance in a wine judging, their liking by consumer and wine experts, and their quality ratings. In addition to basic chemical wine measurements (pH, TA, VA, free and total SO₂), all wines were profiled for their volatile and their macro- and microelemental composition. A headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS) method was developed to determine volatile differences among the wine samples for over 50 different volatile compounds. Inductively coupled plasma–mass spectrometry (ICP-MS) and microwave plasma–atomic emission spectroscopy (MP-AES) were employed to determine the macro- and microelemental differences. Univariate and multivariate statistical methods, including analysis of variance (ANOVA) and principal component analysis (PCA), were used to obtain an overview of sample differences. Canonical variate analysis (CVA) and partial least squares regression (PLS-R) were used to correlate the chemical data of the wines to their previously determined quality ratings and to relate the different data sets to each other. Significant differences among the wines were found using all the methods, and correlations between the quality ratings and the measured parameters will be discussed.

Funding Support: American Vineyard Foundation (no. 1333): Judging Wine Quality

Impacts of Color and Sensory Attributes in Red Wine Varietals

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Red wine color plays a key role in purchase impacts and consumer satisfaction. Three *Vitis vinifera* wines (Cabernet Sauvignon, Merlot, and Zinfandel) were blended using a mixture design model, resulting in 10 wines (three single-component wines, three binary blends, and four tertiary blends). Initial compositional and analytical color components of the wines were analyzed, including identification of anthocyanins by HPLC. Visual color was evaluated by ranking the 10 wines for redness, lightness, and brownness using a consumer panel (n = 70), in addition to descriptive analysis (n = 9) and consumer evaluations (n = 108). Blended wines exhibited similar attributes to their single counterparts. The consumers visually ranked Cabernet Sauvignon more red, more brown, and the darkest, followed by Merlot, then Zinfandel, with red

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Enology — Sensory/Chemistry Session — CONTINUED

color density and percent polymeric color following the same pattern. The primary anthocyanin detected in the wines was malvidin-3-*O*-monoglucoside. Through principal component analysis, three dimensions explained 89% of the variability among the wines. Dimension 1 (71%) was explained by differences between Zinfandel and the other wines, dimension 2 (12%) described differences between Merlot and Cabernet, and dimension 3 (6%) described differences between Merlot blends and Zinfandel blends. Visual redness was positively ($r > 0.90$) correlated to the consumer attribute (appearance), the descriptive attributes (red color intensity, brown tint, depth of color, mouth-feel/body, and spicy flavor), the analytical components (color density, percent polymeric color, and malvidin-3-*O*-(6-acetyl)monoglucoside), but negatively ($r > -0.90$) correlated to the descriptive attribute for clarity and the analytical components (L^* , chroma, and petunidin-3-*O*-monoglucoside). Product appearance was also important for consumer liking. Consumer overall liking for these wines was negatively correlated to hue ($r = -0.87$), cyanidin-3-*O*-monoglucoside ($r = -0.86$), and L^* ($r = -0.78$) and positively correlated to brown tint ($r = 0.69$), visual redness ($r = 0.66$), and percent polymeric color ($r = 0.64$). Optimizing wine appearance is critical to ensuring consumer satisfaction and future purchase intent.

Funding Support: USDA NIFA Specialty Crop Research Initiative

HPLC Determination of Red Wine Tannin Concentration and Prediction of "Grippiness" Following Direct Injection

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Tannins are an integral component of red wine, and are known to impart astringency, a tactile sensation often described in qualitative terms such as "soft" or "aggressive." It is generally accepted among the winemaking community that tannin structure modification plays a role in these descriptors. Based upon isothermal titration calorimetry experiments, the mechanism of astringency is initiated by hydrophobic interaction between tannin and salivary protein. Using this as a basis, a reversed-phase high-performance liquid chromatography method was developed to measure the thermodynamics of interaction between tannins and a hydrophobic surface (polystyrene divinylbenzene) following the direct injection of wine. The consistency of specific enthalpy of interaction in serially diluted wines (-4905 ± 152 J/mol) confirmed that the thermodynamics of tannin interaction with a hydrophobic surface is an intensive property. In addition, the standard addition of purified tannins varying in specific enthalpy of interaction to a red wine confirmed that the specific enthalpy of interaction changed based upon the fractional contribution of wine and tannin standard present in the wine matrix. Tannin fining trials with gelatin indi-

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cated that the specific enthalpy of interaction became less exothermic with an increase in gelatin addition and concomitant reduction in tannin concentration, consistent with observations that protein fining of tannins is preferential for larger tannins. Based upon the developed analytical method, wines from various regions and experiments were analyzed with some comparison to winemaker assessment.

Funding Support: American Vineyard Foundation

New Insights into Managing Microoxygenation

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The use of microoxygenation is widespread in red winemaking. It is generally employed immediately post-yeast fermentation (Phase I) or later during aging (Phase II) as a means to reduce vegetal character and improve color stabilization. However, there are few metrics to monitor the progress of the treatment. Here we investigated both phases, with a Phase I study that compared newly fermented wine that was used (1) as is, (2) sterile filtered, and (3) filtered plus yeast. In stirred, 6-gallon tanks, oxygen was added at 30 mL/L/month for 30 days. The wine was analyzed for oxygen, phenolics by HPLC, and carbonyls. After two days, the oxygen levels in the yeast-inoculated wine fell quickly, and after one year in bottle, the resulting wine was dramatically different from the other treatments and controls (no O₂), showing much lower levels of anthocyanins. The Phase II experiment used filtered wine and compared a control against wines with added SO₂, or glutathione, or both. Using the same equipment, the wine was treated with 15 mL/L/month for 30 days with similar analyses. Here, oxygen levels dropped after free SO₂ was depleted, with rapid increases in acetaldehyde and increases in acetaldehyde acetals and wine pigments. Both experiments suggest factors that may be useful in managing microoxygenation treatments.

Funding Support: American Vineyard Foundation

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Viticulture — Rootstocks Session

Salt Exclusion in Grapevine

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Plant-based strategies for maintaining long-term performance of grapevines in saline environments include grafting with rootstocks that have an ability to limit the uptake and/or root to shoot transport of chloride (Cl^-) and sodium (Na^+). Such rootstocks, by reducing salt accumulation in leaves and fruit, minimize salt effects on scion photosynthetic rates and ensure Cl^- and Na^+ concentrations are not exceeded in domestic and export wines. Research using rooted leaves as a model system has involved genotypes with high (140 Ruggeri), medium (Cabernet Sauvignon), and low (K 51-40) capacity for Cl^- exclusion. The high Cl^- exclusion capacity of 140 Ruggeri appears related to its capacity to sequester more Cl^- in roots, reduce Cl^- concentrations in xylem, and, as a result, limit accumulation of Cl^- in the petiole and lamina. When concentrations of Cl^- on a whole-rooted leaf basis are compared, there is no difference among the three genotypes, indicating the exclusion phenotype involves partitioning of Cl^- within the plant. There was no difference between 140 Ruggeri and K 51-40 in accumulation of Na^+ , indicating that different mechanisms are involved in regulating accumulation of Cl^- and Na^+ in these genotypes. However, the poor Cl^- excluder also accumulated higher concentrations of K^+ . Crossing 140 Ruggeri with K 51-40 to generate a hybrid family ($n = 60$), and assessing the accumulation of Cl^- , Na^+ , and K^+ by the hybrids, revealed a continuous spectrum of variation of all three ions within the family, indicating that accumulation of each ion was controlled by more than one gene. Comparing the root transcriptomes of the parents by microarray hybridization revealed differentially expressed anion transporters and channels, which could mediate the Cl^- exclusion process in 140 Ruggeri.

Funding Support: Grape and Wine Research and Development Corporation, CSIRO, and Adelaide University

*indicates corresponding author

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Viticulture — Rootstocks Session — CONTINUED

Rapid Recovery from Drought Stress in Deeply Rooted *Vitis* Rootstocks

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One way of categorizing the analysis of drought resistance in plants is to examine the division between structure and function. Structural analyses would quantify relevant traits such as root system architecture, root-to-shoot ratio, and leaf cuticle thickness. Functional analyses would alternatively look for dynamic responses to drought stress and are often more physiological in nature, such as measurements of photosynthetic rate and stomatal conductance. We combined these approaches by tracking root and shoot growth responses and stomatal conductance of four grafted rootstock genotypes grown in deep rhizotron containers. After approximately one month of well-watered establishment, controls were watered daily and drought-treated plants were left unwatered until stomatal conductance fell to low values ($\sim 100 \mu\text{moles H}_2\text{O}/\text{m}^2/\text{sec}$). Stressed vines were then rewatered to pot capacity and watered daily thereafter. We used two deep-rooted rootstocks, 110R and Ramsey, known to invigorate the shoot system and produce high yields, and two shallow-rooted, more fibrous rootstocks, 101-14 and Riparia Gloire, known to devigorate the shoot system and sometimes negatively impact yield. Constitutive rooting patterns for all four genotypes followed established trends. Twenty-four hours after rewatering, both deep-rooted rootstocks had reestablished stomatal conductance to levels equivalent to that observed in the well-watered controls. The shallow-rooted Riparia Gloire took 3 to 5 days to recover to well-watered values, and 101-14 never completely recovered even following 7 days of daily watering. Leaf area measurements were uninformative on this time scale, except to document the slow growth of Ramsey, known to occur in young vines. New root growth was significantly higher for drought-stressed 110R and Ramsey during the drought period. This ability to continue higher rates of root production in drying soil may provide a primary explanation for the rapid recovery from drought stress observed in the deep-rooting rootstocks and may provide a useful measure for drought-resistance rootstock breeding.

Funding Support: E&J Gallo, 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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Viticulture — Rootstocks Session — CONTINUED

Differences in the Drought Tolerance Characteristics of Seven Grape Rootstocks

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Drought tolerance is a complex mechanism that involves different traits. Multiple combinations of these traits have been found to confer drought tolerance in different species. This study examined anatomical and morphological features to study the drought tolerance mechanisms utilized by seven grape rootstocks. The goal was to develop an inexpensive, quantifiable screening tool for detecting drought tolerance in new crosses. Three drought-resistant rootstock (Ramsey, 140Ru, and 110R) and four drought-susceptible rootstocks (101-14Mgt, 5C, 420A, and Riparia Gloire) were planted in seven blocks at the UC Davis research fields. Four of the blocks were subjected to drought treatment and three were well irrigated over the two-year duration of the experiment. Each block contained 10 replicates of each rootstock and was completely randomized. Morphological and anatomical measurements revealed innate and responsive trait differences between the two groups of rootstocks. Drought-tolerant rootstocks had significantly deeper rooting angles and allocated more biomass to their roots than drought-susceptible cultivars under well-watered conditions. Total root biomass did not change significantly in drought-tolerant cultivars when exposed to drought versus well-watered conditions. However, drought-susceptible cultivars increased the amount of total root biomass significantly when exposed to drought, suggesting that there is a carbon allocation response to drought among susceptible rootstocks but that drought-tolerant rootstocks are innately prepared for drought stress. Anatomical measurements found that drought-tolerant rootstocks produce a greater number of vessels with smaller diameters (<40 μm) when exposed to drought treatment but not under well-watered conditions. Further analysis confirmed that the lumen area was significantly reduced in Ramsey and 140Ru during the second half of the summer when exposed to drought.

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

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Viticulture — Rootstocks Session — CONTINUED

Optimization of *Vitis aestivalis*-derived Norton Grape Breeding Using Molecular Genetic and Genomic Approaches

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Vitis aestivalis-derived Norton, the official grape of the state of Missouri, is grown in many regions of the United States where *V. vinifera* production requires extensive pesticide use for fungal disease management. Norton has been reported to be cold hardy and resistant to several fungal pathogens, including powdery mildew, downy mildew, *Botrytis* bunch rot, and black rot. In view of this, a mapping population of 184 individuals was constructed from a cross between Norton and *V. vinifera* Cabernet Sauvignon. A haploid Norton genetic map has been constructed with 359 simple sequence repeat (SSR) markers clustered in 19 linkage groups. In collaboration with VitisGen (www.vitisgen.org), approximately 170,000 single nucleotide polymorphism (SNP) markers generated by genotyping-by-sequencing (GBS) have been identified in this population and will be integrated with SSR markers to construct a high-resolution linkage map. In preparation for placing traits on this map, phenotyping assays for powdery mildew, downy mildew, and *Botrytis* bunch rot resistance have been established and will be applied to the population. Careful genetic mapping of this population provides the foundation and tools to associate molecular markers with these three fungal disease resistance traits of Norton. The ultimate goal of this program is to reduce the dependence of viticulture on pesticides and to optimize the production and quality of Norton-based hybrids.

Funding Support: Missouri State University Faculty Research Grant, Missouri Department of Agriculture Specialty Crop Block Grant 12-25-B-1680, and USDA-NIFA AFRI Competitive Grant 2013-67014-21360

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Enology — Winemaking Practices Session

Impact of Mechanical Harvesting and Optical Berry Sorting on Grape and Wine Composition

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With the ever-increasing cost and shortage of qualified labor and the desire to economize vineyard operations, mechanizing the harvest of grapes for wine production has become increasingly important over the past 50 years. Today, a number of companies manufacture self-propelled harvesters that straddle vineyard rows and use rapidly pulsating bowed rods to shake the vines and free berries from the rachis. Despite the high efficiency and economic advantages of these machines, some in the winemaking industry resist their application based on a belief that wines made from mechanically harvested grapes are inferior to those made from hand-harvested fruit. Concerns associated with mechanical harvesting include damage to the berries, the inclusion of materials other than grapes (MOG), increased microbial and enzymatic activity between picking and processing, and loss of valuable juice in the vineyard. It has been proposed by others, however, that coupling mechanical harvesting with optical berry sorting technology may lead to wine that is as good or better than wine made by traditional hand harvesting and sorting methods. In this study, Pinot noir grapes from the Russian River were harvested respectively by hand, by a Pellenc mechanical harvester with a Selectiv' Process on-board picking head, and by the same Pellenc mechanical harvester with the Selectiv' apparatus disengaged. For each harvest method, half of the grapes were unsorted and half were sorted using a Bucher Vaslin Vistalys R1 optical berry sorter. Wines were made from each treatment in triplicate. The composition of the grapes and finished wines was assessed by measuring their phenol and aroma profiles. Wine analyses will be repeated after bottling to coincide with descriptive sensory analysis.

Funding Support: Wine Spectator Scholarship, Napa Valley Vintners

Effect of Regulated Deficit Irrigation on Saignée Treatments of Cabernet Sauvignon Must

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In 2013, the impact of saignée was studied in Cabernet Sauvignon grapes sourced from a 36-year-old, own-rooted vineyard near Mattawa, Washington, subjected to four regulated deficit irrigation (RDI) treatments: (I) 100% replenishment of crop evapotranspiration (100% ET_c), (II) 70% ET_c, (III) 25%

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Enology — Winemaking Practices Session — CONTINUED

ET_c until veraison, followed by 100% ET_c until harvest, and (IV) 25% ET_c. As RDI treatments affect both berry weight and grape phenolic content, we evaluated saignée and juice additions to determine if the berry weight impacts observed in the vineyard treatment could be compensated for in the winery. To do so, each vineyard replicate was made into wine with two replicates designated as controls, and two replicates from each treatment had either saignée or juice addition. Based on berry weight data gathered at harvest, saignée was applied at 20%, 18%, and 10% to the fruit harvested from the I, II, and III vineyard treatments, whereas treatment IV had a 20% juice addition. Anthocyanins were significantly greater in vineyard treatment IV on a per berry basis, while skin and seed tannins were only significantly affected on a fresh weight basis (IV > I, II, III). We found that although the saignée and juice addition treatments significantly impacted wine anthocyanins and tannins to the degree the treatments were expected, they did not exceed the impact of the vineyard treatment. Evaluation of the anthocyanins on a fresh weight basis revealed that treatment IV had 40 to 54% more than the other vineyard treatments, which explains why neither the saignée nor the juice addition changed the pigment concentration enough.

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

Investigation of Pump-over Frequency on Phenolic Extraction and Fermentation Rate during Cabernet Sauvignon Production

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The phenolic content of red wine is responsible for the color, mouthfeel, and ageability of the wine, all of which are important factors in determining its quality and commercial viability. Red wines are fermented in contact with the grape skins and seeds, from which the phenolics are extracted into the wine. During red wine fermentation, the skins form a cap on the surface of the fermentor, reducing contact time between the juice and the skins and also trapping the heat of fermentation. The fermenting juice is pumped over the cap during fermentation so that juice-skin contact is increased and the heat of fermentation is dispersed. Experiments performed during the 2011 and 2012 harvests showed that pump-over volume had little effect on the extraction of phenolics and the rate of fermentation. As these experiments investigated only pump-over volume, little is known as to how pump-over frequency will effect phenolic extraction and fermentation rate. The effect of pump-over frequency on phenol extraction and fermentation rate of different Cabernet Sauvignon

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Enology — Winemaking Practices Session — CONTINUED

fermentations was investigated at the UC Davis Winery during the 2013 harvest. Pump-over frequencies of eight, four, two, and one time per day were investigated while maintaining a constant total pump-over volume of 4 volumes per day. All fermentations were performed in triplicate using automated research scale fermentors with a 120 L working volume and were sampled twice daily. All fermentation samples were analyzed by UV-VIS spectroscopy for color and tannin content and by reversed-phase HPLC to construct fermentation profiles for each pump-over frequency. Finished wines will also be analyzed six months after treatment to determine any differences in phenolic evolution and tannin composition among the treatments during bottle aging.

Funding Support: E&J Gallo Wineries

Effects of Sulfur Dioxide on Fermentation Kinetics, the Wine Microbiome, Prion Induction, and Ester Formation

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Sulfur dioxide is indispensable in modern winemaking; it blocks oxidation reactions and functions as an antimicrobial agent to ensure speedy fermentations and insulate wine against spoilage. Bacteria and non-*Saccharomyces* yeast display differing sensitivities to SO₂. Bacterial blooms in juice can significantly impact the fermentation kinetics and quality of the finished wine, but some wild biota activity may improve wine quality and complexity. The SO₂ concentration can be adjusted to selectively inhibit the microorganisms found in juice. The goal of this research project was to dynamically vary the concentration of SO₂ in triplicate in native and inoculated Chardonnay fermentations and assess the impact of sulfite level on fermentation biota, fermentation progression, induction of yeast prions, and ester formation. Certain strains of common wine microorganisms can, when grown with yeast, induce the [GAR⁺] prion state, which we have shown previously negatively impacts fermentation performance. SO₂ affected the fermentation behavior of wild type and [GAR⁺] yeast as well as strongly influenced the entire microbial population of these fermentations. We also observed differences in the volatile ester production through solid-phase microextraction mass spectrometry, indicative of altered metabolism and stress response in these fermentations and of the potential contributions of the wild biota present in the fermentations. Lower concentrations of sulfur were generally correlated with higher frequencies of [GAR⁺] induction, indicating that at these lower levels, bacterial growth was permissible, allowing them to influence the metabolism and fermentation behavior of the yeast. Our results are important for winemakers to consider when

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making sulfur additions. Varying the levels of sulfur in must can seriously impact the robustness of fermentation, as well as have long-ranging effects on wine quality and stability.

Funding Support: UCD Scholarships and the Government of Japan

Strategies to Prevent Turbidity Problems after the Addition of Carboxymethyl Cellulose to Red Wines

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The addition of carboxymethyl cellulose (CMC) inhibits crystal nucleation and growth, and therefore tartrate precipitation, in the bottle. Commercial CMC products are mostly not recommended for red wines because their interaction with colored pigments might have negative effects on the wine. Besides color loss, intense turbidity could be observed in some red wines several days after CMC addition. The aim of this study was to identify the source of this haze and develop strategies to allow tartrate stabilization by CMC in red wines. Ninety commercial wines made from eight grape varieties were collected, tested with two commercial CMC products, and analyzed for susceptibility to haze formation. Seventy-four of these wines showed precipitation after 7 to 14 days, independent of the CMC product used. Varieties like Pinot noir seem to be more susceptible to haze formation than Cabernet Sauvignon, for example. The precipitate of four representative samples was selected for further investigation and analyzed for elemental composition (CHNS-analysis), solubility under different conditions, and by LC-TOF-MS to determine the composition of the haze. Independent of the sample, the precipitate contained about 50% protein and 50% CMC and polyphenols. Furthermore, the color loss could be explained, with the influencing factors leading to precipitation including the total phenol:anthocyanin ratio, which is responsible for the variety effect. The pH plays an important role only for the precipitation process, not for the protein-CMC interaction. The loss of CMC due to precipitation influenced stabilization in some cases, which made a second addition necessary. However, after the second CMC addition, no further haze could be observed. According to these results, a strategy for CMC addition to red wine with limited risks could be established, including pretests and longer waiting times prior to bottling.

Funding Support: N/A

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Enology — Winemaking Practices Session — CONTINUED

Closure Consistency

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Wine bottle closures have different performance characteristics. A key factor 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 among closures is also quite important, particularly if the variation is high enough to produce two bottles of differently tasting wine. To address this question, a Sauvignon blanc wine was bottled under 200 natural corks, 200 synthetic corks, and 200 screwcaps. The wines were bottled by an experienced mobile bottling company during a standard commercial bottling run. The absorbance of the wine at 420 nm was measured in each bottle quarterly for about 18 months. The browning of the wine correlated well with chemical oxidation and the sensory response to oxidation. The data showed increases in the absorbance of all the bottles. The average change was fairly similar, but natural corks had the greatest variability, synthetic corks the lowest, and screwcaps in between. The level of SO₂ was measured in selected bottles, and sensory evaluation is being planned.

Funding Support: Plumpjack Group

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Viticulture — Grapevine Diseases Session

Managing Powdery Mildew: How Specific Product Use Can Change the Timing of the Critical Period for Intervention

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Grapevine powdery mildew management was revolutionized with the discovery of ontogenetic or age-related resistance in grape tissue. Modern chemical and cultural management programs have shifted focus to this critical period for disease control. From 2012 to 2013, research on the interaction between fungicide timing and fruit-zone leaf removal during this critical period was conducted at WSU-IAREC in Prosser, Washington. Trials consisting of multiple fungicide program rotations paired with four different leaf removal timings were conducted on mature *Vitis vinifera* Chardonnay and Riesling. Results show that the timing of specific fungicide modes of action within a rotation program can significantly impact the final efficacy (disease severity) of that control program ($F = <0.0001, 0.0015, \text{ and } 0.03$ for Chardonnay 2012, Riesling 2012, and Riesling 2013, respectively). In some instances, the timing of fruit-zone leaf removal altered the final disease severity when used within a specific fungicide rotation program; the interaction was more apparent during a season of higher disease pressure (i.e., 2012). This is likely due to increasing exposure risk of the fruit (in a poor, early season program) or enhancing product efficacy by reducing environmental conduciveness for mildew development on the fruit between fungicide sprays (in effective management programs). These findings suggest that disease management during the critical period of fruit infection may be enhanced by the specific, focused use of different fungicide modes of action. In addition, the specific timing of early fruit-zone leaf removal may enhance or detract from that overall disease management, depending on the disease pressure that is a result of the early season fungicide program efficacy.

Funding Support: Washington State Grape and Wine Research Program

Making the Case for Early Adoption of Preventative Practices for Management of Grapevine Trunk Diseases

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Experimental trials on prevention of trunk diseases *Botryosphaeria* dieback, Esca, and *Eutypa* dieback have shown that delayed pruning, double pruning, and applications of the pruning wound protectant thiophanate-methyl (Top-sin) can reduce pruning wound infections by 25 to 75%. Despite this biologi-

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Viticulture — Grapevine Diseases Session — CONTINUED

cal evidence, these practices are not typically adopted by growers until disease incidence reaches levels of >20% in mature vineyards. Possible explanations include confusing disease management guidelines, discouraging results due to late timing, and negative perceptions of disease control efficacy. There are no efficient eradication controls. Our goal is thus to encourage early adoption of preventative practices in newly established vineyards, before trunk diseases start to impact yields. A survey of approximately 350 grapegrowers in five regions of California (Napa-Sonoma, Central Coast, northern San Joaquin, southern San Joaquin, and northern California) was conducted during eight meetings organized by extension agents and regional grower groups, using the audience-response software Turning Point. Respondents answered questions on use of preventative practices (never to always), vineyard age when practice was adopted (0 to 3, 4 to 7, 8 to 12, or 13+ years), and perceptions of disease control efficacy and cost-effectiveness (very ineffective to very effective). In all but one region (Napa-Sonoma), preventative practices were typically adopted in vineyards >8 years old, with 15% of vines showing trunk disease symptoms. Delayed pruning was the most common of all three practices except in Sonoma, where pruning wound protectants were most common. Growers with positive perceptions of practice efficacy and cost-effectiveness tended to adopt the practice in vineyards <8 years old, suggesting that timing these preventative practices before trunk disease symptoms become apparent does maintain adequate yields and is thus cost-effective. With a clear understanding of these trends in usage and grower perceptions, we will develop new extension tools that better communicate the advantages of preventing infection in newly established vineyards.

Funding Support: USDA-NIFA Specialty Crop Research Initiative (grant to KB)

Economic Benefits of Early Adoption of Preventative Management of Trunk Diseases

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Trunk diseases such as *Botryosphaeria* dieback, Esca, *Eutypa* dieback, and *Phomopsis* dieback present a serious challenge to grapegrowers. Despite high disease prevalence and substantial yield impacts, most growers routinely wait to treat until the vineyard is 8 to 12 years old and has a 10 to 40% disease incidence, when vines appear symptomatic and preventative practices have limited efficacy. Delayed pruning, double pruning, and hand-painting applications of the pruning wound protectant thiophanate-methyl (TopsinM) have been effective in experimental trials, decreasing pruning wound infections by 25 to 75%. Nonetheless, growers hesitate using these practices, possibly because they cannot

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use trial-and-error to evaluate their efficacy and/or because economic returns (revenue – cost) from adoption are difficult to predict in a long-lived, perennial crop. Using economic simulations for winegrape production in five California regions (Napa-Sonoma, Central Coast, northern San Joaquin, southern San Joaquin, and northern California), we estimated benefits of adopting these practices under varying disease control efficacy levels (25, 50, and 75%) in 3-, 5-, and 10-year-old vineyards for their remaining lifespan. All but one scenario resulted in greater returns across all regions than those under no action. Also, all practices and efficacy levels evaluated in 3-year-old vineyards resulted in positive returns. No action resulted in negative annual average returns in northern California, northern San Joaquin, and the Central Coast, ranging from -\$476 to -\$1,110 per acre. In these regions, we observed positive annual returns when delayed pruning or TopsinM with $\geq 50\%$ efficacy was adopted in ≤ 5 -year-old vineyards. Returns in southern San Joaquin and Napa-Sonoma remained positive with no action, but fell to 5% (\$89/acre/year) and 10% (\$659/acre/year) of returns expected from healthy vineyards. Lastly, adoption in 3- and 5-year-old vineyards translated into longer positive year-to-year returns, extending vineyard profitability by as much as 5 to 10 years.

Funding Support: USDA-NIFA Specialty Crops Research Initiative

Diversity of Pierce's Disease Vectors East of the Rocky Mountains

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Pierce's disease continues to be a limiting factor in the production of high quality winegrapes throughout the southern United States. The current understanding of vector diversity, distribution, and seasonality responsible for the spread of Pierce's disease by *Xylella fastidiosa* has been primarily a function of work conducted in California, but east of the Rocky Mountains there has been an absence of such critical information. This knowledge is essential in understanding disease dynamics in grapegrowing regions outside of the West Coast and for the development of practical control recommendations. In addition to survey work in other states, work conducted throughout Texas reveals that there are a multitude of putative vector species from at least five tribes of cicadas that occupy various niches across a diversity of ecosystems. In areas previously thought to be free of the disease, once it was identified, additional insect survey work revealed a unique guild of vector species that had not been detected by standardized trapping techniques used to monitor the glassy-winged sharpshooter (*Homalodisca vitripennis*). Insect survey data is beginning to shed light as to why some growing regions appear to be at much higher disease risk than others despite the presence of similar vectors in each. Differences in vector density and regional differences in vector guilds and seasonality of vector distributions are beginning to offer insight into some of these regional

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Viticulture — Grapevine Diseases Session — CONTINUED

differences in disease pressure. Analysis of trapping data also reveals the impact of weather patterns on vector populations and provides insight into some of the reasons this disease has been cyclic over time.

Funding Support: Texas Research and Education Program, USDA APHIS PPQ

***Xylella fastidiosa* Genetics in Texas: Analysis on Multiple Spatial and Temporal Scales**

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Pierce's disease (PD) of grapevines is caused by the bacterial plant pathogen *Xylella fastidiosa* subsp. *fastidiosa* (Xf). The pathogen is vectored into the xylem by sharpshooter insects and the infection kills *Vitis vinifera* vines within two growing seasons. PD has historically been a challenge for grapegrowing in Texas and continues to limit the expansion of vineyards in the state. Starting in 2003, we began investigating the genetics of Xf strains in Texas to improve strain detection and to better understand the epidemiology and evolutionary history of this pathogen. Over the last decade, we have used simple sequence repeat technology to investigate the short-term movement (months, years) of the Xf grape strain throughout Texas. Working with collaborators at USDA-ARS, these investigations suggest relative ease of population movement throughout the state and population separation between Texas and California (with evidence of a few population exchanges). Analysis of two Xf genomes from Texas suggested that the grape strain was highly conserved across the United States and that there was recombination between the grape and nongrape strains (Xf subsp. *multiplex*). Collaborations with UCR have subsequently shed light on how Xf subspecies have radiated in North America (evolutionary time scales) and the role of homologous recombination between subspecies in host shifting. This overview of Xf genetics in Texas builds our understanding of pathogen ecology on multiple spatial and temporal scales.

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Viticulture — Grapevine Diseases Session — CONTINUED

Comparative Sequence Analysis of the Pierce's Disease Resistance Locus *PdR1*

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Pierce's disease (PD) resistance exists in many native grape species from the Gulf Coast states, accessions collected from Mexico, and bordering U.S. states. We have genetically tagged PD resistance from a form of *Vitis arizonica* b43-17 as *PdR1* locus on chromosome 14, with two distinct resistant haplotypes that were termed "a" and "b." This study results from a comparative analysis of the disease resistance region of haplotype "a" and "b" sequences in concurrence to the reference grape genome sequence of PN40024. The BAC library developed from b43-17 was screened to identify clones that represent the *PdR1* locus and a physical map was completed with sequences generated from the Pac Bio SR II sequencing. Preliminary sequence analysis indicates that the *PdR1* locus harbors a family of tightly linked resistance genes in a cluster, with long tandem repeats of transposable elements interspersed among them. Transposable elements are highly variable and are by far the most common genetic elements causing genomic variations in plants. Transposable elements are also associated with disease resistance genes and can allow rapid genetic change. The two haplotypes were compared to the reference grape genome sequence of PN40024 to assess the extent of divergence between resistant and susceptible sequences, to predict gene structure and annotation, and to understand the organization of transposable elements in the region.

Funding Support: CDEA Pierce's Disease Board

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Viticulture — General Session

Response and Recovery of Grapevines to Severe and Persistent Reductions in Irrigation

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The Australian Millennium drought, when below average rainfall across the Murray Darling Basin catchment severely reduced water available for irrigation, resulted in the region suffering its first irrigation drought. The Basin, being predominantly an arid environment, means horticulture is totally dependent on irrigation for profitable production. For irrigators, season-opening irrigation allocations were as low as 0%, and final allocations, 18%. Such severe reductions in irrigation allocations resulted in significant impacts on production, and hence the economic survival of individual properties, communities, and businesses. At the height of ongoing reductions in allocations, winegrape growers began to question the economics of buying increasingly expensive water to maintain yield or their vineyard asset. Of particular concern was first, an understanding of the critical volumes of irrigation water required to produce an economically viable and marketable crop, and second, the long-term consequences of severe irrigation reductions on vineyard survival and recovery postdrought. A long-term study, in collaboration with industry, was established to quantify grapevine response to reductions in irrigation ranging from moderate to extreme over a number of consecutive seasons and to assess yield recovery after these different periods and degrees of water stress. The 11-acre experimental site of drip-irrigated, mature Chardonnay vines grafted to Ramsey rootstock in the South Australian Riverland comprised four replicates of six levels of irrigation and one to four seasons of irrigation restriction. Although record summer rain was recorded midway through the experiment, the impact of the previous seasons of severe irrigation reductions were still evident in later seasons; these and other data will be presented. The range of irrigation reductions imposed enabled the development of a yield response function for irrigation volumes far below levels previously investigated and the development of recommendations for growers on management options for future droughts.

Funding Support: Yalumba Wine Company, Yalumba Nursery, SA Premiers Drought Task Force, Australian Grape and Wine Research & Development Corporation

*indicates corresponding author

Thursday ASEV Oral Presentation Abstracts (Research Reports)

2014 ASEV TECHNICAL ABSTRACTS CONTINUED

Viticulture — General Session — CONTINUED

Long-Term Trends and Epidemiology of Pierce's Disease in Texas

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Pierce's disease, caused by *Xylella fastidiosa*, emerged very quickly as one of the most serious limiting factors to successful winegrape production in central and south Texas. The combination of supplemental hosts for the native pathogen, robust vector populations, including the glassy-winged sharpshooter (*Homolodisca vitripennis*), conducive environments, and susceptible grape varieties make it impossible to produce unprotected grapes with any degree of success. Extremely steep disease progress curves have been recorded for susceptible grape varieties in Texas, and rapid vine-to-vine spread in vineyards is clearly a factor in the epidemiology of the disease. A long list of native plants, many in the Asteraceae, Fabaceae, and Solanaceae families, are suspected as unsafe through artificial inoculations with *X. fastidiosa* subsp. *fastidiosa* and field isolations of the pathogen. However, the issue of inoculum sources is one of the many problems in our understanding of Pierce's disease in Texas that requires further investigation. The risk of Pierce's disease infection decreases from southeast Texas, where only tolerant varieties such as Blanc du Bois and Black Spanish can be grown, to the High Plains where hard freezes, fewer vectors, and lower populations of supplemental hosts reduce the impact of the pathogen on vineyards. Changing climatic conditions appear to be increasing the risk of the disease on the Texas South Plains, the most recent grapegrowing region in Texas in which the pathogen has been discovered. Disease levels elsewhere appear to have diminished during recent years, probably due to the influence of unfavorable climate on vector populations and the widespread adoption of vector management with insecticides. This trend cannot be relied on to diminish the threat of Pierce's disease to the Texas winegrape industry.

Funding Support: Texas Research and Education Program, USDA APHIS

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Viticulture — General Session — CONTINUED

Responses of Phenolic Compounds to Sunlight Intensity in Cool-Climate Cabernet franc

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With the goal of developing light response curves for phenolic compounds in cool-climate Cabernet franc, vines in a New York Finger Lakes region vineyard were treated with four levels of leaf removal, resulting in a range of cluster exposure flux availability (CEFA). Fruit was harvested at maturity, crushed, destemmed, stabilized with sulfur dioxide, and sampled after 48 hr maceration. Samples were analyzed by high-performance liquid chromatography to quantify phenolic compounds. In 2012, the CEFA range was 9 to 60% at fruit set and 17 to 54% at veraison. In 2013, CEFA ranges were 12 to 67% and 13 to 55%, respectively. In 2012, linear regression revealed that phenolic compounds correlated positively with fruit set CEFA (total flavonols: $p < 0.0001$, $R^2 = 0.70$; total hydroxycinnamates: $p = 0.0044$, $R^2 = 0.45$) and with veraison CEFA (flavonols: $p = 0.0004$, $R^2 = 0.61$; hydroxycinnamates: $p = 0.0183$, $R^2 = 0.34$). In 2013, phenolic compounds correlated positively with fruit set CEFA (total anthocyanins: $p = 0.0255$, $R^2 = 0.31$; flavonols: $p < 0.0001$, $R^2 = 0.90$; hydroxycinnamates: $p = 0.0291$, $R^2 = 0.30$; and condensed tannins: $p = 0.0445$, $R^2 = 0.26$). Compounds responding positively to veraison CEFA included anthocyanins ($p = 0.0010$, $R^2 = 0.55$), flavonols ($p < 0.0001$, $R^2 = 0.89$), hydroxycinnamates ($p = 0.0007$, $R^2 = 0.57$), and tannin ($p = 0.0072$, $R^2 = 0.41$). Comparison of fruit set and veraison responses suggested that relative phenolic concentrations at maturity among treatments can be determined as early as fruit set. Quadratic regression suggested that all responses of anthocyanins, hydroxycinnamates, and tannin were nonlinear, with positive responses turning negative when CEFA exceeds maximum response (44% at fruit set, 50% at veraison for hydroxycinnamates in 2012; 47%, 49%, and 50% at fruit set and 40%, 40%, and 41% at veraison for hydroxycinnamates, tannin, and anthocyanins, respectively, in 2013).

Funding Support: USDA/NIFA

*indicates corresponding author

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2014 ASEV TECHNICAL ABSTRACTS CONTINUED

Enology — General Session

Phenolic and Aroma Composition of Grapes and Wines from Five Hybrid Grape Varieties Used in Northern Wine Production

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The northern wine industry has been developing tremendously during the last 10 years. Northern wines are mostly produced from hybrid grapes, including French and cold-hardy varieties, that are complex interspecific crosses of *Vitis vinifera* with *V. riparia*, *V. labrusca*, and other *Vitis* sp. The extended breeding tree of most hybrid varieties gives them specific chemical compositions that make current knowledge on *V. vinifera* varieties of little use to northern winemakers. Therefore, in order to increase our understanding of hybrid grape winemaking, we sampled five significant red varieties (Frontenac, Marechal Foch, Marquette, Sabrevois, and St. Croix) used for wine production in Quebec, Canada, and did an extended analysis of phenolic (hydroxycinnamic esters, flavonoids, anthocyanins, and proanthocyanidins) and aroma profiles (GC-MS-SPME) in grapes and in the corresponding wines. Sabrevois and St. Croix showed the highest levels of hydroxycinnamic esters in the skin (552 and 565 mg caffeic ac. eq./kg berry, respectively) and in the wines (651 and 723 mg caffeic ac. eq./L, respectively). St. Croix showed the highest level of monomeric anthocyanin in the skin (1522 mg malvidin-3-glycoside eq./kg berry) and in the wine (1041 mg malvidin-3-glycoside eq./L). Skin polymeric tannins (186 mg epicatechin eq./kg berry) were much higher in St. Croix compared to other varieties, but St. Croix wines had low tannin level (97.3 mg epicatechin eq./L), with monomers (26% of total tannins), trimers (20%), and octamers (16%) dominating over other species. Aroma profiles of Marechal Foch and Sabrevois juices showed marked levels of ethyl butyrate (106 and 259 ppb, respectively) and ethyl 2-butenate (26.8 and 40.4, respectively), which both correlated significantly with more ethyl 3-hydroxyhexanoate in the respective wines. Marquette showed the highest level of monoterpenes in both grapes and wines, with linalool, β -citronellol, and geraniol being the main monoterpenes found in the wines, at 29, 25, and 20 ppb, respectively.

Funding Support: Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec (MAPAQ), Canada

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Enology — General Session — CONTINUED

Supramolecular Sensors for Fingerprinting Red Wines

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Incidents of wine adulteration have given substantial impetus to developing new techniques for the verification of wine authenticity. One factor that contributes to the uniqueness of individual red wine varieties is the composition of a class of polyphenols known as tannins. Tannins are known to contribute two characteristics to red wine character: astringency and bitterness. As a platform for an electronic tongue, here we describe the development and application of peptide-based supramolecular sensing ensembles for discrimination of red wines. Differential binding of tannins to the receptors causes indicator displacement, which can be measured spectrophotometrically. Absorbance change in response to wines was analyzed using multivariate statistics protocols such as principal component and linear discriminant analyses. By using an array of peptides, divalent metals, and colorimetric indicators, red wines were clustered and classified based on varieties and, in some cases, different vintners. In addition, an array of nine ensembles was used to successfully discriminate a panel of Cabernet Sauvignon wines made from grapes that were harvested at different stages of maturation. To further demonstrate the power of this technique, our sensing ensembles were used to examine the contribution of individual wines to the character of blends that comprise different ratios of the monovarietals. While certain blends appeared to exhibit a response comparable to the predominant base wine, others displayed an unexpected pattern. Further analysis of responses to the sensing array and sensory attributes of wine revealed a strong correlation of the peptide receptors to perceived astringency. This approach emphasizes the utility of differential sensing to fingerprint complex mixtures without identifying all single components.

Funding Support: Howard Hughes and NSF

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Enology — General Session — CONTINUED

Rapid, Inexpensive Headspace Method for Determination of Molecular Sulfur Dioxide

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Sulfur dioxide (SO_2) is the primary antioxidant and antimicrobial preservative used in commercial winemaking. The measurement of the antioxidant form (free SO_2) is traditionally performed by the Ripper or aeration-oxidation (A-O) methods, from which the concentration of the antimicrobial form (molecular SO_2) is derived from the sample pH and the pK_a of SO_2 . These traditional measurement approaches require sample dilution and/or pH shifts, resulting in partial hydrolysis of bound bisulfite adducts and overestimation of both free and molecular SO_2 . We describe a fast, simple, and inexpensive static headspace sampling method for determining molecular SO_2 in wine samples. Briefly, a syringe is partially filled with a wine sample, the wine and its headspace are allowed to equilibrate, and the headspace is then forced through a colorimetric SO_2 gas detection tube. The headspace SO_2 concentration can then be related to the molecular SO_2 concentration of the wine using Henry's Law. Because no dilution or acidification step is employed, molecular SO_2 determined by this approach reflects the actual molecular SO_2 of the sample. The volatility of SO_2 (Henry's coefficient) was constant over ethanol concentrations of 0 to 17% v/v, and only the temperature of samples needed to be adjusted prior to analysis. Reasonable agreement between new and classic methods was achieved for molecular SO_2 in white wines. However, in red wines, molecular SO_2 determined was ~50% of that by A-O or Ripper, likely because of dissolution of weakly bound bisulfite-flavylum adducts in these classic methods. Additionally, calculations are complicated because industry-accepted pK_a values for SO_2 do not account for effects of alcohol, temperature, and ionic strength, which may underestimate the molecular SO_2 by more than half. Ongoing work is investigating whether headspace molecular SO_2 measurements are a better predictor of microbial stability than classic methods.

Funding Support: New York Grape and Wine Foundation and the Canandaigua Wine Endowment Fund

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Viticulture — Grape Maturity Session

Effect of Light Environment on Methoxyypyrazine Content of Cabernet Sauvignon

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Methoxyypyrazines (MPs) are compounds responsible for vegetative aromas in various winegrape varieties. Vegetative aromas include asparagus, peas, and green bell pepper. These compounds are detected at low sensory thresholds. At low concentrations, MPs can add positive varietal character; however, higher concentrations of MPs can be undesirably herbaceous and diminish fruity aromas. Previous research has shown a relationship between light exposure and MP content in fruit and wine. However, heat is an effect of light, so it is difficult to separate the effects of these two factors. The focus of this study was to separate heat and light by utilizing a novel light-emitting diode (LED) treatment. LEDs emit light without excessive heat, thereby allowing the separation of these factors. Field experiments in 2011, 2012, and 2013 exposed both green developing fruit and postveraison fruit to three treatments: (1) full sun, (2) full shade, and (3) full shade with supplemental LED light. Treatments were established by positioning shoots. Fifteen vines were selected for each treatment, with one cluster per vine receiving a treatment. In the field, photosynthetically active radiation (PAR) data were repeatedly measured. Clusters were collected 50 days postanthesis and postveraison when Brix was approximately equal to 24. 3-Isobutyl-2-methoxyypyrazine (IBMP) and 3-isopropyl-2-methoxyypyrazine (IPMP) were measured in fruit from each treatment. To quantify MPs, stir-bar sorptive extraction-gas chromatography-mass spectrometry (SBSE-GS-MS) was used. In 2011, 2012, and 2013 preveraison and postveraison, the shade treatment PAR was lower than both full sun and LED. With the exception of 2012 preveraison and 2011 postveraison, LED PAR tended to be less than full sun. For 2012 data, IBMP in preveraison shaded fruit was greater than both sun and LED, but all treatments degraded by postveraison. IPMP was greatest in shaded fruit postveraison.

Funding Support: Texas A&M AgriLife Research and Extension

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Viticulture — Grape Maturity Session — CONTINUED

Development of a Grape Chemical Quality Scale for *Vitis vinifera* L. Cabernet Sauvignon

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Historically, the perception of grape quality is influenced by source region and relies predominantly on sensory characterization of fruit in the vineyard. Past efforts to relate grape chemical composition to finished wine quality have brought to light a number of chemical compounds that may be effective markers for estimating the quality of winegrapes according to the sensory properties of color, aroma, taste, and mouthfeel. Over a period of five years, Cabernet Sauvignon grapes from vineyards across major California winegrowing regions were chemically characterized and investigated for relationships between chemical composition of fruit and sensory quality assessment of resulting wines. Strongest candidate measures, including total anthocyanins, skin and seed tannins, organic acids, yeast assimilable nitrogen, isobutyl methoxypyrazine, glucosyl-bound norisoprenoids, and total C_6 -alcohols, were modeled to create standardized scores that provide a means to gauge relative grape quality across vineyards. The distribution of chemical quality scores reveal trends in grape quality both within and across regions, which may reflect differing climatic conditions, soil types, and viticultural practices.

Funding Support: E&J Gallo Winery

The End of Ripening

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In contrast to widespread and intensive studies on physiological and genetic aspects of the onset of ripening, characterization of the end of ripening has received little attention. Lang and Thorpe (1989) described the postveraison berry as “a small bag of sugary water,” and Lang and During (1991) provided some evidence that cell compartmentation is lost from veraison onward. In this study, we report on cell viability, berry water budget, and sugar influx during development to generate a hypothesis for the end of ripening. Cell viability determined by fluorescence of fluorescein diacetate revealed a late onset of cell death in berry mesocarp beginning approximately 110 days after anthesis (DAA). Analyses of berry growth, water relations, and total soluble solids were combined into a model of xylem flux, phloem flux, and total sugar per berry. Data from several varieties were collected from vineyards in Davis, California, and other data were taken from the literature to generate the model. The re-

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Viticulture — Grape Maturity Session — CONTINUED

sults of the model indicated that for Cabernet Sauvignon, sugar accumulation ceases approximately 110 DAA and phloem influx, shortly thereafter. Some varieties were similar and others differed in the timing of cessation of sugar accumulation; e.g., earlier in Shiraz. The cessation of sugar accumulation was better predicted by DAA than by a final Brix or GDD, suggesting that the rate of ripening in this climate is not heat dependent. The results are discussed in the context of alternative definitions for the end of ripening.

Funding Support: California Agricultural Experiment Station

Impact of Cultural Practices on Cold Hardiness Dynamics of Six *Vitis vinifera* Cultivars

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Grapevine winter injury is a major limitation for premium wine production in marginal climates such as Ontario, Canada. To produce some premium wine styles in Ontario such as sparkling wine, appassimento-style wine, and icewine, various crop levels and/or harvest dates are required to ensure product typicity without compromising vine cold hardiness. Replicated factorial experiments of two cropping levels × multiple harvest dates were imposed on three white cultivars (Chardonnay, Sauvignon blanc, and Riesling) and three red cultivars (Pinot noir, Cabernet franc, and Merlot) over a 3-year period. Crop level treatments consisted of two cluster-thinning levels of 1 cluster/shoot and 2 clusters/shoot. Harvest of these treatments occurred at normal commercial maturity or with an extended hang time of an additional 3 weeks. Vine performance parameters, yield components, and fruit composition were compared to cold acclimation/deacclimation curves. In midwinter, there were few differences in cold hardiness among treatments but some among cultivars. However, crop level and harvest date impacted rates of acclimation and deacclimation for most cultivars. There were also vintage effects due to cold hardiness dynamics and interactions of crop level × harvest date. Full crop × late harvest treatments resulted in slower vine acclimation, and crop levels had a greater impact on cold hardiness dynamics of Chardonnay, Pinot noir, and Sauvignon blanc. Maximum cold hardiness may be influenced more by cultivar specificity based on rates of maturation than by crop load or harvest date. Growing season conditions and timing of maturity also play an important role in terms of the

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Viticulture — Grape Maturity Session — CONTINUED

interactions between crop levels, hang time, and cold hardiness. Smaller crop treatments and late harvests were characterized by lower yields, higher soluble solids and pH, and lower titratable acidity. Therefore, by reducing crop levels and extending hang time, greater fruit quality can be achieved, but vine cold acclimation may be delayed for some cultivars.

Funding Support: Ontario Grape and Wine Research, Ontario Ministry of Economic Development and Innovation's Ontario Research Fund, Agriculture and Agri-food Canada's Developing Innovative Agri-Products Initiative

Improving Freezing Tolerance of *Vitis vinifera* Pinot gris Grapevines Using Exogenous Abscisic Acid

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Grape and wine industries in colder regions such as Ohio have been expanding rapidly, and demand for premium winegrapes has also increased. However, several popular cultivars are sensitive to freezing temperatures below -20°C. The goal of this study was to improve freezing tolerance of sensitive grape cultivars using abscisic acid (ABA). Previous work indicated that exogenous ABA application increased freezing tolerance of cold-sensitive cultivars. The objective of this research was to evaluate another cold-sensitive cultivar, Pinot gris, by evaluating yield components, fruit composition, and freezing tolerance in response to exogenous ABA. Field-grown Pinot gris vines were sprayed with 400 mg/L ABA at different stages of development (veraison, postveraison, and postharvest). ABA did not affect yield components or fruit composition, but caused early leaf abscission, advanced bud dormancy, and eventually increased freezing tolerance. Veraison and postveraison treatments were most effective in increasing freezing tolerance. The findings of this study are valuable to grape producers by providing another tool for freeze protection and to the scientific community by providing better understanding of the mechanisms of freezing tolerance.

Funding Support: Department of Horticulture and Crop Science, OSU; USDA-NIFA; Ohio Grape Industries Program

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Enology — Wine Chemistry Session

Elemental Profiling of Malbec Wines from Argentina and California Made under Controlled Winemaking Conditions

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The use of elemental fingerprints has been widely accepted to differentiate among geographical origins for many foods and beverages, including wines. However, there have been recent studies showing that strictly controlled experiments need to be performed to determine true geographical origin tags, so that processing, storage, shipping, etc., are not adding to the elemental profile and then driving the observed differences. In this study, we evaluated the elemental profile of Malbec wines made with grapes from 26 Argentinian and 15 Californian vineyards and processed under controlled winemaking conditions. A bench-top microwave plasma atomic emission spectrometer (MP-AES) was used for the elemental analysis of the wines. This instrument provides a cost-effective tool for elemental profiling, particularly compared with more expensive ICP-OES or ICP-MS instruments. The elemental differences between, and especially within, Argentina and California can be traced back to the geographical location, as the winemaking was done in the same facility. Within each set (Argentinian and Californian), there were wines from several wine regions, and all the wines were made in one of two facilities (one in Argentina, one at the University of California, Davis). Therefore, the observed differences among the wines should be attributed to the different vineyards. Observed differences among the samples will be discussed with regards to potential sources. The use of an MP-AES as an alternative to other spectroscopic methods for elemental measurements will be highlighted as well.

Funding Support: The Catena Zapata family and participating U.S. vineyard owners and winemakers

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Profiling the Chemical Composition of Semillon Grape Berries at Three Stages of *Botrytis cinerea* Infection

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Botrytis cinerea is a necrotrophic plant pathogen that is particularly aggressive on ripe fruit. Under specific environmental conditions, infections of winegrape berries by *B. cinerea* cause “noble rot,” characterized by physicochemical modifications of infected berries that contribute to the unique properties of botrytized wines. Postveraison grape berries at three stages of infection (Stages one to three) by *B. cinerea* were harvested from the Dolce Winery vineyards in Napa

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Enology — Wine Chemistry Session — CONTINUED

Valley. Asymptomatic grape berries were collected as controls. The berries were deseeded, frozen, and ground in liquid nitrogen on the day of harvest. Samples were analyzed by reversed-phase UHPLC/QTOF MS, and the QTOF MS was run in negative mode with a mass range of 100 to 1700 *m/z*. The samples were also analyzed in a data-dependent, automated MS/MS mode to support identification of compounds found in the samples. Analysis of the MS data produced a set of entities that was used to differentiate among berries from the different *B. cinerea* infection stages. Asymptomatic berries were readily differentiated from infected berries even at very early stages of infection (Stage one). More advanced stages of infection (Stages two and three) were partially separated, but well differentiated from stage one infected berries. While the identification of the specific plant metabolites induced by *B. cinerea* infection is ongoing, several classes of compounds that appear to be increased include anthocyanins, flavonoids and their glycosides, as well as monoterpenes and lipids. The results indicate that noble rot activates plant metabolic pathways (e.g., anthocyanin synthesis) that are not present during the normal ripening process of Semillon grape berries. This study is part of a multidisciplinary “-omics” approach using transcriptomic data to prioritize the metabolomic analysis. The metabolomic and transcriptomic data will be integrated to characterize metabolic pathway regulation.

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

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

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Winemakers often add exogenous products to increase tannin content in red hybrid wines, but the recommend dosage of 50 to 400 mg/L may not effectively increase condensed tannins. In 2013, wines were made from Maréchal Foch, Corot noir, and Cabernet franc to compare the retention of exogenous tannins in interspecific hybrids and *Vitis vinifera*. For each cultivar, Ferco Grap'tan, with a condensed tannin concentration of 38%, was added at a rate of 400, 800, and 1200 mg/L after crush and before yeast inoculation. One lot of each cultivar was also pressed and the juice fermented with 1600 mg/L of Grap'tan, then back-blended postfermentation with a control wine for a final theoretical concentration of 400 mg/L. After cold stabilization, tannin concentration for the 400 mg/L addition was different in Maréchal Foch and Cabernet franc wines, showing an increase of 29.2 and 64.6 mg/L, respectively. Additions of 800 and 1200 mg/L resulted in lower final tannin concentrations in Maréchal Foch than in Corot noir and Cabernet franc (72.2, 123.8, and 120.4 mg/L at 800 mg/L additions and 129.8, 219.3, and 244 mg/L at

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Enology — Wine Chemistry Session — CONTINUED

1200 mg/L, respectively). The back-blended wines were not different than those with 400 mg/L additions in Corot noir and Cabernet franc but showed higher tannin retention in the Maréchal Foch (29.2 and 62.2 mg/L for the 400 mg/L and back-blended additions, respectively). Though concentrations of condensed tannins were higher in all wines, none were as high as expected, given the measured concentration in Grap[®]Tan additions. This study suggests that high concentration additions of exogenous tannin increases the condensed tannins in hybrid red wines but not to the same extent as equivalent additions in wines produced from red *V. vinifera* cultivars.

Funding Support: USDA Specialty Crops Research Initiative

Impacts of Ion Exchange Treatment on Wine Flavor Chemistry

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High pH and titratable acidity (TA) wines are becoming more prevalent due to irrigation practices, climate change, and adoption of new cultivars. This is problematic because high pH wines are microbially unstable, and acid addition risks detrimentally affecting wine flavor balance. A possible solution is the use of cation exchange, which selectively removes buffering compounds, i.e., potassium (K⁺), replacing it with H⁺ ions to lower the pH. This process has not been widely adopted due to concerns that it will deleteriously impact wine flavor. Limited research has been done to understand these concerns. An aromatic white (Valvin Muscat) and two red wines (Norton and Chambourcin) were passed through a column filled with Amberlite IR 120 (H⁺) cation exchange resin until depletion. Percent alcohol, pH, TA, K⁺, Ca²⁺, Mg²⁺, volatiles, total anthocyanins, total phenolics, color density, and oxidative stability were quantified before and after treatment. The ion exchange resins were also analyzed posttreatment to confirm potential compound losses. The pH of the ion-exchanged wine decreased by an average of pH 0.7 while the TA was unaffected. The ion exchange treatments resulted in a 0.2% reduction in ethanol and a 10% reduction in total anthocyanins. Preliminary GC-MS results indicate a reduction in ethyl decanoate in Chambourcin. No significant difference was found in the concentrations of several esters and higher alcohols across all wines. The treatments resulted in a 70% reduction in K⁺, Ca²⁺, and Mg²⁺.

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Enology — Phenolic Chemistry Session

Controlling Oxidation of Model Wine Using Metal Chelators

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Nonenzymatic oxidation of wine can be deleterious to its quality, particularly in the case of white wines. At present, winemakers have few ways to control or prevent oxidation processes. In this study, the use of exogenous Fe(II) (bipyridine; Ferrozine) and Fe(III) chelators (ethylenediaminetetracetic acid, EDTA; phytic acid) in wine was studied as a means to control nonenzymatic oxidation. The formation of primary oxidation markers, 1-hydroxyethyl radicals (1-HER) and acetaldehyde, were measured using electron paramagnetic resonance (EPR) and HPLC-PDA, respectively. In addition, the chelators were investigated for their ability to prevent the oxidative loss of a varietal thiol, 3-mercaptohexan-1-ol (3MH). The Fe(II) chelators were more effective than Fe(III) chelators during the early stages of oxidation, as seen by the decrease in oxidation markers. However, Fe(III) chelators proved to be more effective antioxidants than Fe(II) chelators over the long term. In addition, it is shown for the first time that iron chelators can significantly inhibit the oxidative loss of 3MH in model wine.

Funding Support: Pennsylvania State University Food Science Department and Frederik Sr. and Faith E. Rasmussen Endowed Professorship in Food Science

Impacts of Grape Maturity and Ethanol Concentration on Wine Tannin Concentration

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Seed tannins are largely believed to be problematic by winemakers, who believe that longer ripening and higher ethanol concentrations in the must contribute to altering the extraction of seed tannins during red wine production. Seed tannins are known to decline during grape ripening; however, as riper fruit will potentially yield a greater ethanol concentration and potentially more seed tannin extraction, there remains a conundrum. In this work, we sought to understand how grape maturity and ethanol concentration impact wine tannins. In 2013, we carried out three separate harvest dates for our experiment, representing underripe (20 Brix, DOY 248), ripe (24 Brix, DOY 268), and overripe (28 Brix, DOY 305) fruit. For each harvest, two-thirds of the must was adjusted to emulate the soluble solids of the other harvests to evaluate the effect of ethanol on phenolic extraction at different maturities (saignée and/or waterback to maintain juice to solids ratio). The resulting wines had 11.8, 13.9, and 16.2%

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Enology — Phenolic Chemistry Session — CONTINUED

(v/v) ethanol. We found that only wines with 16.2% (v/v) ethanol had a significant impact on tannin extraction, which was independent of harvest date. We also found that the wines made from overripe fruit had significantly greater wine tannins than wines from the other harvests, which were not different from each other. Thus, waiting for overripe fruit did not result in less tannic wines. In conclusion, as yeast have different ethanol tolerances, we found that ethanol concentrations unachievable by *Saccharomyces cerevisiae* but instead by *Saccharomyces bayanus* had a significant impact on tannin extraction. Thus, within the ethanol concentrations achievable by *Saccharomyces cerevisiae* there is little risk of greater tannin extraction via ethanol from 20 to 28 Brix fruit.

Funding Support: Washington Wine Grape Funds and Washington State University

Investigating the Effect of Cold-Soak Duration on the Extraction of Phenolics during Cabernet Sauvignon Fermentations

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Cold-soak treatments are an enological technique employed in the fermentation of red wines with the intent to maximize the extraction of water-soluble compounds such as anthocyanins and polysaccharides from the grape prior to the onset of alcoholic fermentation, when less water-soluble compounds, such as tannins, are extracted. The length of these cold-soak periods may last from 4 to 7 days at temperatures ranging from 10 to 15°C. In general, prior research on cold-soak during fermentations has focused on Pinot noir fermentations and how the finished wine is affected by the cold-soak period and not on phenol extraction kinetics. Additionally, little is understood about how the cold-soak treatment affects the polysaccharide content of the finished wines or how different red wine varieties will be affected by cold-soak treatments. A greater understanding of the extraction chemistries during the cold-soak period, how different lengths of cold-soak time affect phenolic and polysaccharide extraction, and how these extractions are reflected in the finished wine would provide winemakers with the information needed to use cold-soak treatments effectively with different red wine varieties. To accomplish these tasks, Cabernet Sauvignon fermentations were performed with 1, 4, 7, and 10 days of cold-soak treatment. During the cold-soak period, samples were taken regularly so that the extraction chemistries of the phenolics and polysaccharides could be investigated by reversed-phase (RP)-HPLC and LC-MS/MS. Samples taken during active fermentation were also analyzed to determine if any changes seen during the cold-soak period persist during fermentation and how

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the cold-soak period affects phenolic extraction kinetics during fermentation. The phenolic and tannin profiles and polysaccharide content of the finished wines were measured to determine whether the different cold-soak treatments had a lasting effect on wine composition and quality.

Funding Support: E&J Gallo Wineries

Sorption of Grape Proanthocyanidins and Wine Polyphenols by Inactivated Yeast Fractions

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Inactivated yeast fractions (IYFs) are inactivated yeasts or yeast fractions proposed as aids in enology as alternatives to aging on lees to improve the mouthfeel of red wines. However, information concerning the mechanisms involved and the impact of IYF characteristics (strain, soluble versus insoluble fractions, process) is scarce. One possible mechanism of action is the sorption of red wine polyphenols by IYFs. We compared the sorption of polyphenols by solid IYFs obtained from an enological yeast strain cream/biomass (Y) through different processes: drying (inactivated yeast, IY), autolysis and drying (autolyzed yeast, AY), and mechanical disruption (cell walls, CW). The impact of the process on IYF structure was assessed by transmission electron microscopy. Surface charge, hydrophobicity, and acid/base character of the yeast (under cream form, Y) and the IYFs (IY, AY, and CW) were determined by electrophoretic mobility measurements and the microbial adhesion to solvent (MATS) method. Their interactions with grape seed and skin proanthocyanidins and with a total red wine polyphenolic pool were studied in a winelike solution using adsorption isotherms. Results indicated high affinities of Y, IY, and AY toward grape proanthocyanidins, along with high sorption capacities. Although preferentially adsorbed in comparison to nonpolymeric polyphenols, wine tannins exhibited much lower affinities for biosorption than grape tannins. Considering the electron-donor character of Y and YDPs, this can be attributed to chemical changes in tannin structure associated with winemaking. Drying and autolysis decreased the affinity of the yeast cell surface for grape tannins but increased it for wine tannins. This was associated with differences in the surface hydrophobicity and electron-donor character of Y and IYFs. By contrast to what was observed with Y, IY, and AY, polyphenol adsorption on cell walls obtained by mechanical disruption remained very low.

Funding Support: INRA/SUPAGRO

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Viticulture — Vineyard Practices I Session

Comparison of Bilateral Cordon Training Methods on the Development and Productivity of Grapevines

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For cool, windy climates and/or lower-vigor site situations, delays in vine development during training can result in a greater number of growing seasons to achieve full crop yield potential. Training strategies used can be a critical factor in promoting vine development and production that are appropriate to the site conditions. Bilateral cordon training methods conducted in the second year after planting were evaluated for their influence on growth and productivity during the early years of vine establishment. These experiments were conducted in a Chardonnay vineyard and in two Pinot noir vineyards in the Salinas Valley. Cordon training methods compared were: (1) traditional bilateral cordon, (2) traditional with cordons trained vertically on a string, (3) unilateral cordon plus a lateral in year two, and (4) unilateral cordon plus a lateral in year three. All sites were designed as randomized complete block experiments. At the Chardonnay site, in which only treatments 1 and 3 were evaluated, a unilateral trained cordon plus a lateral resulted in larger vines that had higher yield potential in the early production years. For the two Pinot noir sites, even though a unilateral trained cordon plus a lateral resulted in slightly larger vines, a smaller growth response was seen as compared to the Chardonnay site. In the Pinot noir sites, there were no significant yield increases during the early production years in the experimental cordon training methods when compared to the traditional cordon training.

Funding Support: Monterey County Vintners and Growers Association

Under-Trellis Management Impacts Agrochemical and Nutrient Leaching in a Finger Lakes Vineyard

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The rapidly growing *Vitis vinifera* grape industry in the Finger Lakes region of upstate New York is concentrated on the hillsides directly adjacent to the lakes, raising concern of pollution from runoff and leaching of nutrients and agrochemicals. The standard vineyard floor management practice in the region is to allow permanent vegetation to grow between rows, and to maintain an herbicide strip approximately one meter wide under the trellis. Cover crops grown under the trellis have the potential to reduce erosion, improve soils, and limit pollution of local watersheds. Four under-trellis management systems were established in a Cabernet franc vineyard in Lansing, New York, in 2009:

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glyphosate herbicide (GLY), cultivation (CULT), native vegetation (NV), and white clover (*Trifolium repens*) seeded annually at 10 lb/acre (WC). Drainage lysimeters were installed beneath the under-trellis treatments to monitor nutrient and pesticide concentrations in leachate water samples. In 2012, nitrogen leachate concentrations from the WC treatment were 349% and 281% greater than that of NV and CULT, respectively; nitrogen leachate concentrations from the GLY treatment were 150% and 112% greater than NV and CULT, respectively. In 2013, GLY nitrogen leachate concentrations were 573% and 464% greater than that of NV and CULT, respectively. The bare soil treatments of GLY and CULT had similar dissolved, organic carbon concentrations to one another, and were greater than dissolved organic carbon concentrations in the cover-crop treatments of NV and WC in both years. In 2012, imidacloprid insecticide was found in measurable concentrations in 28% of GLY leachate samples and imidacloprid metabolites in 33% of samples; in contrast, only 6% of CULT and WC and 0% of NV samples contained imidacloprid at measurable concentrations or its metabolites. These results suggest that some cover crops may influence leaching of nutrients and pesticides from the vineyard.

Funding Support: USDA SCRI

Evaluating Crushed Glass Mulch, Dried Distillers Grain, and Groundcovers for Sustainable Vineyard Floor Management

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Our objectives were to identify alternatives to glyphosate for in-row vineyard floor management and to evaluate the potential for in-row and alleyway groundcovers to reduce vegetative vigor of Marquette vines in a southeast Nebraska vineyard. The experiment was a randomized factorial design with five in-row treatments (crushed glass [CG], distillers grain [DG], creeping red fescue [CRF], unsprayed control, and glyphosate) and three alleyway treatments (creeping red fescue [CRF], Kentucky bluegrass [BG], and resident vegetation). Treatment plots were established in 2010–2011 and measurements were conducted during 2012 and 2013. Soil temperature was higher under mulches and lower under in-row groundcovers compared to glyphosate. Groundcovers generally had lower in-row soil moisture than glyphosate; soil water content was higher under DG but lower under CG in 2013. Treatment effects on vine vigor were inconclusive. None of the treatments differed in vine water potential or shoot length. BG and CRF alleyway treatments had higher pruning weights in 2012 than resident vegetation. Leaf layer number was higher in DG in-row plots, relative to glyphosate. Yield and fruit composition results were also inconclusive. In 2012, the treatments did not differ in yield, berry weight, or Brix. In 2013, CG and SC had higher Brix than CRF, and

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Viticulture — Vineyard Practices I Session — CONTINUED

there were significant alleyway in-row treatment interactions for yield and berry weight. In 2013, CRF and unsprayed control had lower pH than the other in-row treatments. TA did not differ between treatments in either year. This study supports a growing body of evidence that maintenance of a weed-free strip under mature vines is unnecessary in many vineyards, although additional research is needed before making recommendations based on these results.

Funding Support: Fox Run Farms (Brainard, NE) (in kind), University of Nebraska Agricultural Research Division, Nebraska Grape and Wine Board

Effect of Herbicide Strip Width and Late-Season Weed Competition on Winegrape Vine Growth, Berry Quality, and Yield

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Viticulture in the southeastern United States is limited by excessive vigor, high humidity, and a challenging growing environment, all contributing to lower-quality fruit. The objective of this study was to determine effects of five vegetation-free strip widths on vine growth, berry quality, and yield. The study was conducted in 2011 and 2012 on Cabernet franc clone 312 on 101-14 MGT rootstock in the Yadkin Valley region of North Carolina. 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 with paraquat and glufosinate and managed throughout the growing season both years. At veraison, maintenance in half of each treatment ceased and the plot was allowed to grow in native vegetation to determine the effect of late-season weeds on berry quality and yield. In 2011, a decrease in Brix and increase in titratable acidity was observed with increasing VFSW. Also in the same year, late-season weedy treatments had higher Brix than all other treatments. Cluster weights in 2012 increased with increased VFSW. No significant difference in yield was observed among VFSW treatments in 2011 or 2012.

Funding Support: USDA/NIFA Specialty Crop Research Initiative

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Viticulture — Vineyard Practices I Session – CONTINUED

Evaluating Buckwheat and Chicory as Undervine Cover Crops in Northeastern Riesling

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Given the rising concerns of herbicide resistance, runoff, and environmental contamination, coupled with increased consumer desire for sustainably produced wines, investigating ways to eliminate herbicide use in vineyards is warranted. In the northeastern United States, vegetation is traditionally kept between rows, but undervine rows are sprayed with herbicide. In 2012–2013, cover crops were established annually in undervine rows of a Riesling (clone 110/9)/3309C vineyard in Lansing, New York. Buckwheat (*Fagopyrum esculentum*) at 348 lb/acre and chicory (*Cichorium intybus*) at 5 lb/acre were planted in the 1-m undervine row and compared to a weed-free strip maintained with glyphosate. In both years, no differences were found among treatments in predawn or midday stem water potentials, shoot length, or growth rates. Chicory reduced occlusion layers in the canopy and shoot diameter by 1 mm and doubled cluster exposure flux availability in 2013. Chicory reduced yield per vine by 27% and berry size by 13% in the second year of the study. Nitrogen content (% DW) of petioles at veraison was reduced 0.1% by buckwheat treatments in both years and by chicory in 2013. The chicory treatment reduced titratable acidity by up to 1.0 g/L in 2012 and reduced YAN by 35 ppm in 2013 compared to the control. Wine aromas from 2012, tested using multi-dimensional sorting analysis with 53 panelists, were found to be different among undervine treatments. Results indicated that using buckwheat as an undervine cover crop for two years did not impact vine growth or yield, while chicory reduced excessive vegetative growth, yield, and titratable acidity. Further testing of different undervine cover crop species and developing a better understanding of how vineyard floor management choices affect wine aromas in northeastern Riesling will help provide grapegrowers with sustainable alternatives to herbicide.

Funding Support: Ronni Lacroute Dean's Discretionary Fund for Enology and Viticulture at Cornell University, Northeast SARE Graduate Student Grant

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Enology — Wine Tannins Session

Investigating the Gross Structure of Condensed Tannins by Small Angle X-Ray Scattering Analysis

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The gross structure of condensed tannins found in grapes and extracted into wine influences the astringent properties of wine. For example, tannin interactions with salivary proteins involved in astringency is governed by the availability of binding sites on the tannin polymer, which is influenced by polymer tertiary structure. The tertiary structure of tannins is in turn determined by interflavan linkage, subunit composition and sequence, and branching within a polymer. It is also influenced by components of the wine matrix such as ethanol that determine tannin solubility. The aim of this study was to isolate tannins with different structural characteristics and investigate their gross structure using small angle X-ray scattering analysis (SAXS). Tannin polymers found in winegrapes that increase in size from monomer to octamer were isolated from cacao beans using preparative hydrophilic interaction chromatography (HILIC), while tetrameric polymers were further separated using semipreparative C18 liquid chromatography to isolate three structurally different tetrameric isomers. Three-dimensional models of each tannin polymer in aqueous solution were generated following analysis by SAXS. Analysis of the three tetramer isomers demonstrated the ability of SAXS to distinguish tannin isomers likely differing in interflavan bond position. Further investigation will determine the effect of different components of the wine matrix such as ethanol, sugar, and anthocyanin on tannin structure.

Funding Support: Grape and Wine Research and Development Corporation

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Enology — Wine Tannins Session — CONTINUED

Impact of Individual and Mixed Condensed Tannin Polymers on Their Ability to Precipitate Protein

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Numerous methods exist for the measurement of tannins, as their importance in numerous foodstuffs is legion. In red wine, they are important for their astringent properties and their ability to interact with pigments to form polymeric pigments. Protein precipitation has been used to measure tannins in red wines because of its simplicity and strong correlation with sensory results. However, as tannins are a heterogeneous class of compounds and those found in wine grapes are particularly complex, questions have arisen about which specific tannins are measured as part of a protein precipitation assay. To this end, less complex tannins from cacao (*Theobroma cacao*, L.) seeds, which are composed of epicatechin from monomer to octamer, were isolated by preparative-scale HPLC. Once purified, the tannins were added to bovine serum albumin (BSA) individually and combined as mixtures. When added to excess BSA, all of the condensed tannins displayed a linear precipitation pattern. The amount of tannin necessary to precipitate BSA varied among the polymers, with the trimer requiring the most to precipitate BSA (1000 µg) and the octamer requiring the least (50 µg). The efficacy of condensed tannins for protein precipitation increased with increased degree of polymerization (or size) from trimers to octamers. Mixtures of two polymer sizes primarily had an additive effect that was predictable. We studied monomers and dimers over a large concentration range and found they did not precipitate BSA but when added to larger tannin polymers, no additional precipitate was formed. The results of this study show that only polymers of three subunits or more will be counted in protein precipitation assays and that simple mixtures behaved predictably. Further research to expand our understanding of astringency perception and its correlation with protein precipitation would benefit from sensory analysis of condensed tannins across a range of polymer sizes.

Funding Support: Victorian Department of Environment and Primary Industries, Washington Wine Grape Funds, Washington State University, Constellation Brands US, and Grape and Wine Research and Development Corporation (Australia)

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Enology — Wine Tannins Session — CONTINUED

Effect of Condensed Tannin Size on Tannin-Protein Interactions

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In wine, the precipitation of salivary proteins by condensed tannins is associated with the sensory characteristic of astringent perception. The intensity of astringent perception has been reported to be greater at larger polymer sizes, while different subunits are thought to influence descriptors of astringency. The aim of this study was to investigate the interaction of purified tannin structures of increasing molecular weight with protein to gain a better understanding of how tannin structure influences tannin-protein interactions that might be involved in determining astringency. The binding affinity of tannin-protein interactions was measured using isothermal titration calorimetry (ITC), as it quantitatively measures the heat change during molecular reactions (i.e., thermodynamics) and can be used to correlate thermodynamic properties with structural and physical influences of binding. Purified tannin polymers of increasing molecular weight from trimer to octamer were allowed to interact with the protein bovine serum albumin (BSA). It was found that molecular weight had a major impact on tannin-protein binding. The change in enthalpy increased with polymer size but plateaued after the hexameric polymer, while the number of tannin polymer binding sites on the protein decreased as the molecular weight increased. It was also found that both temperature and pH had an effect on tannin-protein interactions.

Funding Support: Grape and Wine Research and Development Corporation

*indicates corresponding author

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2014 ASEV TECHNICAL ABSTRACTS CONTINUED

Viticulture — Vineyard Practices II Session

Influence of Vine Vigor and Crop Level on Pinot noir Vine Growth, Nutrition, Fruitfulness, and Fruit Composition

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Oregon Pinot noir vineyards are characterized as having high vegetative vigor and low yields, resulting in low crop loads, which are exacerbated by cluster thinning. A study was conducted from 2011 to 2013 to evaluate the impacts of vegetative vigor and crop level on growth and fruit composition of Pinot noir. Vine vigor was modified using a combination of perennial grass and tillage in the alleys. Two crop levels (no thinning and one cluster per shoot) were imposed within each floor management treatment. Floor management treatments effectively altered vegetative vigor, as the presence of the grass (*Grass*) flanking the vine row reduced shoot growth, vine leaf area at bloom and veraison, lateral shoot growth, and dormant pruning weights during 2011 to 2013 compared to vines with alternating grass and tillage or complete tillage. The decline in vine size (23 to 46% reduction in leaf area at veraison) was attributed to a reduction in nitrogen concentration in leaf blade and petioles, measured at bloom and veraison. Volumetric soil moisture did not differ with floor management in 2012 and 2013. Decreased soil moisture was seen in *Grass* during summer 2011, but this did not decrease midday stem water potential. Yeast assimilable nitrogen concentration of fruit was 53 to 66% lower in *Grass* compared to other treatments, reflecting the reduced vine nitrogen status. Despite increased solar radiation in the canopy, *Grass* had decreased fruitfulness (12 to 22%) in 2012 and 2013 and yield (18 to 43%) in 2011 and 2013. Cluster thinning created a range of leaf area to yield ratios, which were positively related to total soluble solids and berry anthocyanin concentration and negatively related to tannin concentration. Results of this work suggest that altered nitrogen status of vines proportionally reduced vine growth and yield, attaining similar vine balance.

Funding Support: Oregon Wine Board, and USDA-ARS CRIS project #5358-21000-047-06

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Viticulture — Vineyard Practices II Session — CONTINUED

Vegetative Compensation Response of a Procumbent Grapevine Cultivar under Mechanical Canopy Management

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A trial in the San Joaquin Valley of California investigated how the interaction of pruning systems and mechanical shoot thinning affected canopy performance, yield components, fruit phenolic composition at harvest, and production efficiency of a procumbent cultivar in a warm-climate grapegrowing region. Two pruning systems and three shoot thinning treatments were arranged factorially in a randomized complete block design with four replications. The pruning methods were applied by either handpruning to a target of 25 nodes/m or mechanically hedging and retaining a 100-mm spur height. The shoot density treatments were applied mechanically at a modified E-L scale, stage 17, to retain 40 or 45 shoots/m of a row, or left unthinned. The contribution of count shoots to total shoots increased when mechanical box pruning replaced spur pruning. The contribution of percent count shoots to total shoots was greatest with 40 shoots/m and unthinned treatments. The percent photosynthetically active radiation (PAR) transmission and percent canopy gaps increased with mechanical box pruning and also with the decrease in shoot density per meter of row. Berry and cluster size decreased with mechanical box pruning application. However, because mechanically box-pruned vines carried more clusters, yield per meter of row increased. There was a quadratic response to shoot thinning where berry skin phenolics, anthocyanins, and tannins decreased with the 45 shoots/m treatment when compared with 40 shoots/m and unthinned treatments. Pruning weight per meter of row and leaf area to fruit ratio decreased, whereas Ravaz index (kg yield/kg pruning weight) increased with mechanical box pruning. Shoot-thinning treatments did not affect pruning weight per meter of row or leaf area to fruit ratio. Increasing the amount of PAR and percent canopy gaps by shoot thinning resulted in vegetative compensation from a sparsely populated grapevine canopy, thereby negating its purported effects. The 40 and 45 shoots/m treatments repopulated the canopy rapidly with noncount shoots, thereby increasing the pruning weight per meter of row at the end of the season. In the absence of a physiological response, shoot thinning in a procumbent cultivar is not recommended. Mechanically box pruning to a 100-mm spur height and slowing vegetative growth by irrigating to 50% of daily evapotranspiration variance between fruit set and veraison have resulted in a Ravaz index window (5 to 10 kg/kgL1) and is recommended for procumbent red winegrape cultivars for the region with similar or better berry skin phenolic accumulation than spur-pruned vines.

Funding Support: American Vineyard Foundation

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Viticulture — Vineyard Practices II Session — CONTINUED

Optimizing Fruit-Zone Leaf Removal Practices in Cabernet franc and Petit Verdot

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Shaded canopy fruit zones aggravate disease management and fruit quality can be impaired. Fruit-zone leaf and lateral shoot removal is commonly used to correct this problem; however, only general leaf-removal recommendations exist for the principally grown varieties. Two post-fruit-set leaf/lateral removal treatments (MEDIUM, HIGH removal), one prebloom leaf/lateral removal treatment (P-B), and a no-leaf removal control (NO) were compared to determine their impacts on cluster compactness, components of yield, and fruit composition in Cabernet franc and Petit Verdot. We hypothesized that P-B removal would loosen clusters, reduce yields, and improve fruit composition in both varieties. In fact, P-B removal loosened Cabernet franc clusters by 16% and Petit Verdot clusters by 10%, compared to NO removal. P-B removal reduced crop yield by 46% and berry weight by 6% compared to NO removal, and MEDIUM reduced yield by 26% and berry weight by 4% compared to NO removal in Cabernet franc; P-B removal reduced crop yield by 40% and berry weight by 19% compared to NO removal in Petit Verdot. HIGH and P-B removal increased pH and reduced titratable acidity compared to NO removal in Cabernet franc; HIGH leaf removal reduced soluble solids and titratable acidity compared to NO leaf removal in Petit Verdot. Cabernet franc berry anthocyanins did not differ between NO, P-B, and MEDIUM removal treatments; however, the MEDIUM removal treatment had a 19% greater anthocyanin concentration than did the HIGH removal treatment. Treatment did not affect anthocyanins in Petit Verdot. P-B removal increased total berry phenolics by 16% and 22% compared to NO removal in Cabernet franc and Petit Verdot, respectively. Preliminary results illustrate that prebloom fruit-zone leaf/lateral removal can reduce crop yield and berry weight, loosen clusters, and lead to higher berry phenolics, perhaps due to longer seasonal fruit exposure or smaller berries.

Funding Support: Virginia Wine Board

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Industry

Use of a Noninvasive and Nondestructive Technology to Measure Oxygen and Carbon Dioxide in Wine

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The objective of this study was to validate a laser spectroscopy technology in the gaseous phase used by L.Sensor.O₂ for noninvasive and nondestructive measurement of oxygen in glass and plastic containers. The L.Sensor.O₂ instrument includes an optical/mechanical component which positions the container for analysis and an electronic component for the control interface and analysis software. The measurement is achieved using a low-power, class 1 laser which passes a beam through the headspace of the container. The instrument is able to measure the percentage of O₂ in the headspace, the ppm of dissolved O₂, total pressure inside the container, partial pressure from CO₂ and the content of dissolved CO₂ in g/L. The technology allows the L.Sensor.O₂ to perform the measurement independent of the color of the container. The L.Sensor.O₂ is particularly safe to operate, as it does not require the container to be opened or pierced, thereby removing the risk of bursting a container. The test cycle is fast, repeatable, and simple to operate. The L.Sensor.O₂ requires no consumable components and has no wear parts. The technology replaces other techniques which require either prior preparation of the container to be tested or the use of an invasive probe. The L.Sensor.O₂ allows the operator to significantly increase the frequency of samples tested and eliminates the variability associated with manual, destructive test techniques. The system is portable, allowing it to be placed in the production area for fast, reliable monitoring of the oxygen that enters the container during the filling and capping process.

Funding Support: Enartis Vinquiry and Arol Group

Acadian LSC, a Commercial Extract of *Ascophyllum nodosum*, Improves Brix Uniformity in Pinot noir Winegrapes

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Acadian *Ascophyllum* extracts (AAE) have been used in winegrape production to improve plant establishment, rachis stretch, increase set, and enhance yields. In addition to these responses, visual improvements in color uniformity and wine quality have been observed following applications of AAE, potentially indicating increased uniformity of maturity. The objective of this study was to evaluate the effects of AAE on Brix uniformity of Pinot noir grapes grown on the Central Coast of California. A commercial Pinot noir vineyard in Soledad, California, was selected for this trial. Large blocks were paired by rootstock and scion clone and assigned to either a grower standard practice or AAE treatment. AAE treat-

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ment consisted of a full-season application program at recommended rates and timings. In 2012, samples were taken approximately one month before the commercial harvest. In 2013, samples were taken immediately before commercial harvest. Berry weight and Brix levels were measured on individual berries from different positions within bunches. Cluster weight was greater for AAE-treated bunches; however, average berry weight was the same between treatments, as were pH and average Brix. When individual berries in a bunch were measured for Brix, AAE-treated bunches showed less variation. In 2012, this reduction in variation in Brix came primarily from fewer berries with very low Brix measurements in the AAE-treated fruit, while in 2013 there were fewer berries with very high Brix levels. These differences may be due to differences in sampling time. These results indicate the potential to extend applications of AAE beyond improving rachis length, berry set, and yield to increasing the uniformity of berries both within bunches and across vines.

Funding Support: Acadian Seaplants Limited

New Approach for Studying Oxidation Processes in Wine: An EPR Spin-Trapping Automated Assay

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Wine oxidation is a major problem occurring during winemaking or storage and is due to the oxidation of phenolic compounds and the subsequent development of undesirable flavors. The resistance of wine to oxidation is related to careful control of oxygen and metal ion levels and the presence of antioxidants to prevent formation of free radicals, which are the key intermediates in this process. Electron paramagnetic resonance (EPR) was used as a new tool for quality control to detect free radicals and to determine the level of free radicals in wine during forced oxidation at elevated temperatures and different storage conditions. Special reagents called spin traps, phenyl-*N*-tert-butyl nitron (PBN) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), were used to trap free radicals during the forced oxidation assay. The EPR forced oxidation profile provides an analytical measure for evaluating the overall antioxidant status of wine at each stage of production. The EPR data is also useful for predicting the shelf life of a final product before it goes through costly packaging and distribution. The sample is heated to 70°C to force its oxidation, and the spin trap is added to capture free radicals as they form. Bruker has developed the e-scan bench-top EPR spectrometer, interfaced with an automatic sample changer, to provide EPR oxidation profile data for up to 20 samples per assay period. A typical assay is performed over one to three hours for 10 to 20 samples. The data suggest that EPR is a sensitive method for measuring and improving the resistance of wine to oxidation.

Funding Support: Bruker BioSpin Corporation

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Enology

Free Amino Acids and Volatile Composition of Malbec Must and Wines from Different Regions of Mendoza, Argentina

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The purpose of this work was to evaluate the incidence of the geographical origin on the free amino acids and volatile profile of Malbec musts and wines from Mendoza, Argentina. To this end, grapes of three representative vineyards of Mendoza were studied: La Libertad (Rivadavia, 673 m asl), Agrelo (Luján de Cuyo, 950 m asl), and Gualtallary (Tupungato, 1450 m asl). The wines were elaborated in triplicate according to standard protocols. Volatile compounds were analyzed by HS-SPME and GC-MS, and the free amino acids determination was performed by precolumn derivatization with FMOC and HPLC-DAD. The multivariate statistical techniques (principal component and canonical discriminant analyses) were applied to evaluate the chemical differentiation of the three viticultural regions with respect to amino acid profile. Our results showed significant differences in free amino acids between samples from the three regions studied. Proline was the most abundant amino acid in musts from Agrelo and Gualtallary and in wines from all regions, and alanine showed a similar trend for musts from Rivadavia. We did not observe significant differences in the total free amino acids from Agrelo and Rivadavia, and these levels were higher than those found in Gualtallary. Chemometric study allowed us to obtain a perfect prediction (100%) of must and wine samples according to alanine, arginine, asparagine, cysteine, and glutamic acid. Qualitative and quantitative differences in the volatile composition were observed in all samples studied. Rivadavia and Agrelo musts did not show statistical differences with respect to the total volatile composition, while in wines this behavior was observed for Rivadavia and Gualtallary. Wines from Gualtallary presented minor concentrations of esters, terpenes, and norisoprenoids than the other regions. In conclusion, we demonstrated that geographical origin had a great effect on the amino acid and volatile composition, and this could significantly influence the sensory characteristics of the products.

Funding Support: Instituto Nacional de Tecnología Agropecuaria (INTA), project PNAIyAV 1130043

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Postbottling Development of Sulfur-like Off Aromas in Wines with Added Flavorants

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The occasional appearance of low molecular weight thiols and sulfur-like off aromas (SLOs) in wines during anaerobic bottle storage is a well-recognized fault, but the appearance of this condition in flavored wines has not been previously reported. We evaluated the potential of 16 flavoring agents to form SLOs following their addition to wine and bottle storage. The flavors were spiked into a neutral wine, stored in a low-oxygen environment, and evaluated at one, three, and five months. Two of the flavored wines exhibited SLOs and detectable H₂S after three months. Copper-fining treatment and GC-O retention indices indicated that the odiferous compound in one of the wines was likely an intermediate molecular weight thiol. Identification of the compound and its mechanism for formation is ongoing. We also attempted to develop an accelerated reduction assay to predict if flavoring agents would form SLOs during anaerobic bottle storage. Eight reducing agents were evaluated, of which tris(2-carboxyethyl)phosphine (TCEP) was determined to be most appropriate, in part because it did not have interfering aromas. Both of the flavoring agents that formed SLOs during bottle storage also evolved SLOs and H₂S following TCEP treatment, indicating that the approach may be appropriate for predicting if wine additives will form SLOs during storage.

Funding Support: New York Wine & Grape Foundation

Effect of Coinoculation on Chambourcin Wine Quality

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French-American hybrid grapes, including Chambourcin, grow considerably well in the Eastern United States due to their annual production reliability and adaptability to the cooler, humid climates found in this region. Chambourcin is a naturally acidic variety and potentially exhibits sour and astringent characteristics in varietal wines. Interest in managing acidity in red varieties is a key concern of many Eastern U.S. producers. Research on coinoculation (i.e., the simultaneous inoculation of *Saccharomyces cerevisiae* and malolactic bacteria to the must) has shown the potential to alter wine acidity in addition to increasing production efficiency and reducing the potential for microbial spoilage by eliminating the gap between primary and malolactic fermentations. However, research is limited on the use of coinoculation for red hybrid grape processing. This study investigated the use of coinoculation as a viable method for increasing the quality of wine vinified from Chambourcin grapes (20.8 Brix,

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3.35 pH, 9.01 g/L tartaric acid). Fermentation kinetics were monitored by temperature, decreasing Brix value, and yeast/bacteria culture counts. Additionally, an HPLC method was developed to monitor the degradation of malic acid. Potential, perceptible differences in wines were evaluated using standardized sensory evaluation. Growth of *S. cerevisiae* during primary fermentation did not differ between treatments. Concentrations of *Oenococcus oeni* from the point of inoculation to completion of malolactic fermentation were reduced by $-1.1 \log$ cfu/mL in the control treatment in contrast to an increase of $-0.2 \log$ cfu/mL in the coinoculation treatment. Populations of *O. oeni* at the completion of malolactic fermentation were statistically different ($p < 0.05$) between treatments. No statistical differences ($p < 0.05$) were found in wine pH, titratable acidity, alcohol, volatile acidity, final malic acid concentration, cold stability, and total tannin content between treatments.

Funding Support: Pennsylvania Wine Marketing and Research Board

Effect of Yeast Strain on Appassimento Wine Production in Ontario

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Appassimento wines are made following the process of drying grapes postharvest. Appassimento wine has the potential to become a signature style for the Ontario wine industry, as its production may be used as a tool to combat the difficulties associated with winemaking in a cool climate due to seasonal variations that can impact fruit maturity. Grapes that are dried postharvest contain high starting concentrations of sugar. Therefore, wines vinified from dried grapes can expose the yeast to hyperosmotic stress, which in turn, may affect the sensory properties of the finished wine due to higher production of volatile compounds such as acetic acid and ethyl acetate during fermentation. Hence, the effects of different yeast strains on Appassimento wine production were studied. This project aims to determine the upper limits of *Saccharomyces bayanus* by means of investigating and comparing the different fermentation kinetics of the *Saccharomyces cerevisiae* strain EC118 and the Brock isolate of *S. bayanus* yeast strains at four different Brix levels (control [-21.5], 24.5, 26.0, and 27.5 Brix). Metabolites of interest are acetic acid, ethyl acetate, acetaldehyde, reducing sugars, glycerol, nitrogen, and ethanol production during fermentation. Preliminary data demonstrates that *S. bayanus* is a lower acetic acid producer by approximately two-fold. Preliminary data also shows that both yeasts have the ability to ferment the high-sugar wines to dryness (<5.0 g/L) in the treatments studied, illustrating their feasibility for use by the wine industry. It has been determined that *S. bayanus* takes longer to ferment wines to dry; however, all other kinetics follow similar trends. This project will contribute to knowledge transfer to the industry in order to optimize the appassimento winemaking process.

Funding Support: Brock University, ORF

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Enology – CONTINUED

Fingerprinting Wine Blends Using Indicator Displacement Assays

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Wine blends are formed from monovarietal wines, or wines that differ in their grape type. Winemakers combine monovarietal wines to produce blends with enhanced flavors. As a consequence of this practice, wine blending has been associated with fraud cases for a long period of time. Therefore, there is an increased need for developing sensing techniques that discriminate between wine varieties and blends and to verify their authenticity. Differential sensing is a powerful technique for fingerprinting complex mixtures without necessarily having to identify the individual components of the mixture. We have developed a peptide-based sensing ensemble for fingerprinting wine varieties based on their tannin composition. The receptors are composed of a short peptide, a divalent metal ion, and a colorimetric indicator. This method is based on displacement of the indicator by the analyte (tannin), causing a visible color change. In this investigation, peptidic-sensing ensembles were constructed for the purpose of fingerprinting samples of red wine blends. The wine blends were mixtures of Cabernet Sauvignon, Cabernet franc, and Merlot monovarietals. Using three peptides synthesized in the Freshman Research Initiative Supramolecular Sensors Lab, WAHEDEFF, WEEHEE, and FHFPHHF, peptidic sensors were created. Multivariate data analysis protocols such as linear discriminant analysis were used to analyze the data. Our assays show that the blends were discriminated based on the composition of each monovarietal.

Funding Support: HHMI, NSF, College of Natural Sciences

Comparative Analysis of Wines from *Carica papaya*, *Hibiscus sabdariffa*, and Palm Wine with Two Commercial Brands (White and Red)

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Palm wine and the extracts of *Carica papaya* and *Hibiscus sabdariffa* were fermented with appropriate wine yeast to 12% alcohol, aged for nine months, and analyzed to ascertain their suitability for use as table wines and eventual use. Sodium (Na), potassium (K), magnesium (Mg), copper (Cu), lead (Pb), zinc (Zn), cadmium (Cd), aluminum (Al), manganese (Mg), and iron (Fe) were analyzed using atomic absorption spectroscopy while organic acids, flavonoids, and anthocyanin were analyzed by gas chromatography. Lead and cadmium were absent in all the samples. The palm wine (white wine) had the highest metal concentration; Na, K, Cu, and Zn were the least in the *Carica papaya* (white wine); the least Mg and Al were from commercial red and commercial

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white, respectively; and the least Mn and Fe were from *Hibiscus sabdariffa* (red wine). Total organic acid and total flavonoids in the wines were in the decreasing order of commercial white, commercial red, *Hibiscus sabdariffa*, *Carica papaya*, and palm wine. Total anthocyanin were of the decreasing order of *Carica papaya*, commercial red, commercial white, *Hibiscus sabdariffa*, *Carica papaya*, and palm wine. *Carica papaya* wine had the highest total phenolic compounds while commercial red had the least. Panelists that tested the wine samples could not differentiate the locally produced wines from the commercial grape wines. These wine sources could be developed to industrial ventures.

Funding Support: self

Understanding the Factors Underlying Chronically Difficult to Ferment Juices

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The incidence of stuck or sluggish fermentations can be generally avoided by selecting a suitable yeast for the specific juice composition and maintaining permissive growth. However, there are juices referred to as “difficult to ferment” that chronically display sluggish fermentations that are not rectified by nutrient supplementation or yeast strain selection. These juices may contain a nutrient deficiency not currently addressed by available yeast nutrients or an inhibitor of yeast growth and fermentative ability. Juices were assessed for metal or ion deficiencies or toxicities. The ionic composition of the difficult to ferment juices from several commercial wineries was evaluated. The levels of calcium, magnesium, sodium, potassium, manganese, and zinc were measured using atomic absorption spectroscopy. The levels of the ions were found to be vineyard specific, and although ionic profiles displayed some variation between the difficult juices and the easy juices, the levels present were above those found to be deficient in the following synthetic juice studies, indicating mineral deficiencies are not likely to explain the difficulty in fermentation. The effect of varying levels of vitamins, minerals, and biotin in synthetic grape juice was used to evaluate the impact on fermentation to identify conditions mimicking the fermentation profiles found in the difficult to ferment juices. Four commercial yeast strains (Allegro, D254, EC1118, and Montrachet) with varying nitrogen requirements and fermentation abilities were used in this study. Allegro displayed the highest micronutrient requirement. In contrast, Montrachet showed a significant impact on fermentation only under conditions of multiple micronutrient deficiencies. EC1118 was sensitive to biotin levels, but D254 displayed a higher vitamin level sensitivity. Metabolomic analysis of the yeast from difficult to ferment juices showed an increased accumulation of mannitol, an indicator of reactive oxidative stress, which was confirmed by addition of oxidative stressors to synthetic and actual grape juices.

Funding Support: American Vineyard Foundation

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Descriptive Sensory Analysis of Wines Produced from La Crescent Grapes

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The La Crescent grape, a cross of Elmer Swenson selections St. Pepin and ES 6-8-25 (*Vitis riparia* x Muscat Hamburg), is a cold-hardy cultivar gaining popularity throughout the Midwest. Little information is available regarding the aroma profile of wines made from the grape. The goal of this research was to identify descriptive terms to characterize wines produced from the varietal. La Crescent grape juice from a commercial Iowa winery was used to produce research lots of wine in 2011 and 2012. Descriptive sensory analysis of dry and off-dry versions of the La Crescent wine, a commercial La Crescent wine, and a commercial Moscato wine was conducted. Eleven trained panelists evaluated sample attributes using a 15-cm line scale. Six aroma and flavor descriptors were evaluated in 2011 and seven in 2012; intensity of sweetness and acidity was also evaluated. The intensity of aroma and flavor attributes was measured separately in year 1 and as a combined response in year 2. Grapefruit, lychee, pineapple, and rose were common descriptors to sensory panels for both vintages. The most intense aromas in all 2011 La Crescent wines were grapefruit and pineapple. Grapefruit was the most intense flavor in the dry and off-dry La Crescent wines, while apricot was the most intense flavor in the commercial La Crescent and commercial Moscato wine. In 2012, all wines were characterized by intense rose and lychee aromas. Principal component analysis accounted for 59% of the variability in 2011 and 63% of the variability in 2012; wines were mainly separated by sweetness and acidity on the first principal component. Grapefruit, pineapple, rose, and lychee were the most intense aromas/flavors expressed in dry, off-dry, and commercial La Crescent wines and are important in describing wines made from La Crescent grapes.

Funding Support: Midwest Grape and Wine Industry Institute

Yeast Diversity and Composition in Spontaneous Fermentations of Two Varietals at Three Canadian Wineries

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Spontaneous fermentations are relatively rare among wineries in North America, with winemakers choosing inoculated fermentations to ensure a consistent, reproducible product. However, spontaneous fermentations are known to produce more complex wines, as they allow a diversity of yeast species and strains to participate in fermentation. There is currently a lack of knowledge

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about yeast communities in spontaneous fermentations between white and red varieties. The objective of this study was to compare species/strain diversity and composition between Pinot noir and Chardonnay spontaneous fermentations at three Canadian wineries. Three fermentation vessels were sampled for each variety at each winery. Samples were taken from each tank at four stages of fermentation, as defined by Brix levels. A total of 96 yeast isolates were obtained from each vessel during the fermentation. Non-*Saccharomyces* yeast isolates were identified to the species level by sequencing the D1/D2 domain of the yeast's rDNA. *Saccharomyces cerevisiae* isolates were identified to the strain level using microsatellite loci. Our results indicate that for both varieties, *Hanseniaspora uvarum* was the dominant non-*Saccharomyces* yeast at two of the three wineries, whereas *Pichia* spp. was dominant at the remaining winery. Chardonnay fermentations, as compared with those of Pinot noir, contained a higher percent relative abundance of *S. uvarum* during the mid and end stages of fermentation at all three wineries. Furthermore, Pinot noir fermentations had a higher percent relative abundance of indigenous *S. cerevisiae* as compared with Chardonnay fermentations at all three wineries. Overall, the species/strain diversity of both Pinot noir and Chardonnay fermentations appeared to be similar; however, each winery had a unique composition of commercial and indigenous *S. cerevisiae* strains for each variety. This is likely due to differences in winery location and in inoculation history at each winery.

Funding Support: Quails' Gate Estate Winery and Natural Sciences and Research Engineering Council (NSERC) Collaborative Research Development grant CRDPJ 406796-10, UBC internal wine grant, NSERC Engage Grant EGP 453327-13, UBC Okanagan Biology Dept., and Agriculture Canada Investment Plan

Understanding Carryover of *Vitis labrusca* Associated Aromas in *Vitis vinifera* Wines

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Methyl anthranilate and 2-aminoacetophenone are volatile compounds present in certain *Vitis labrusca* grapes, such as Concord and Niagara. Methyl anthranilate is responsible for the distinct grapy aroma of Concord and 2-aminoacetophenone is responsible for the “foxy” character of Niagara. For the vast majority of *Vitis vinifera* wines, these aromas are not expected to be present at any detectable level; however, in recent years, there has been anecdotal evidence in northeastern United States wine regions of these native notes in wines prepared solely from *V. vinifera* varieties. Methyl anthranilate and 2-aminoacetophenone also have low sensory thresholds (200 µg/L and 0.5 to 2 µg/L, respectively); therefore, even trace levels of these compounds in *vinifera* wines are perceptible. The sources and modes of carryover of foxy/native aromas into *vinifera* wines are currently unknown, and we hypothesized that the major mechanism

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by which *vinifera* wines accumulate *labrusca*-associated character is through aroma scalping by winery equipment during juice and wine processing. Due to the fact that *V. labrusca* and *V. vinifera* varieties are commonly processed in the same facilities in the northeastern United States, certain materials used during winemaking may be capable of transferring these volatile compounds between wines and juices. In order to test this, procedures were developed to access the ability of various polymeric resins widely used during production to scalp methyl anthranilate and 2-aminoacetophenone from model juice and wine solutions. Following scalping, those resins were subjected to unspiked model solutions and their desorption was measured. It was found that not only could methyl anthranilate and 2-aminoacetophenone be scalped by these common polymeric resins but that they could also be desorbed from resins by introduction to a fresh, unspiked model solution. These findings provide a plausible mechanism for the inadvertent introduction of native aroma compounds into wines produced solely from *V. vinifera* varieties.

Funding Support: The Pennsylvania State University

Effect of Pre- and Postfermentation Fining with Polyvinylpyrrolidone on the Aromatic Profile of a Viognier Wine

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Polyvinylpyrrolidone (PVPP) is a synthetic, insoluble polymer that is typically added to wine to increase the chemical stability of the final product. Fining with PVPP improves the color stability of white wine by forming hydrogen bonds between the hydroxyl groups of low molecular weight polyphenols in the wine and carbonyl groups on the PVPP polymer, which results in adsorption and precipitation of the compounds responsible for defects (e.g., browning, pinking). It is unknown whether volatile compounds can be removed from wine as a result of a similar reaction with PVPP. The primary objective of this study was to evaluate the impact of fermentation versus postfermentation PVPP fining on the chemical stability and aromatic profile of a white wine. It was hypothesized that treatment with PVPP before or after fermentation would confer equal benefits of chemical stability to the wine, but the postfermentation treatment would have a deleterious effect on the volatile aroma composition, whereas prefermentation treatment would produce a wine with an aromatic profile and sensory properties comparable to the untreated control. In this study, white Viognier (*Vitis vinifera* L. cv. Viognier) wines were treated with 30 g/hL PVPP before or after fermentation. The effect of the time of PVPP addition on phenolic content, color stability, and volatile aroma profile of each wine were compared to a nontreated control wine. Both the prefermentation and postfermentation treated wines contained significantly less total phenolics and less browning pigments than the control wine ($p <$

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0.05). The aromatic profiles of each treatment were compared before and after fermentation using GC-MS. A better understanding of the differences that can arise between preventative and curative PVPP fining will allow winemakers to make processing decisions that will yield consistent and desirable products for consumers.

Funding Support: Pennsylvania State University

Impact on Agronomic Parameters in Vines and Wine Quality of Foliar Treatments with Yeast Derivatives

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The use of yeast derivatives in the wine world has been limited to its use in winemaking. This study tried the application of wine yeast derivatives on vines. Through foliar treatments in a commercial vineyard, the impacts on grape composition and final wine quality were studied. The tests consisted of the application of yeast derivatives specifically designed to be used with the patent foliar application technology WO/2014/024039. The response of plants was evaluated, studying parameters such as accumulation and distribution of dry matter, leaf water potential, yield components, and must quality. Through sensorial analysis, the impact of treatments on the final quality of the wine was assessed. Treatments on cv. Shiraz using RD-LM product were carried out to study the effects on polyphenols concentration. Treatments on cv. Sauvignon blanc using RD-LA product were carried out to evaluate possible effects on aromatic and thiolic fractions of the wines. The parameters were studied at three different times of harvest. The results showed a greater significance due to harvest date than to the treatments. Although in both treatments, the must composition was not affected in the same way, either qualitatively or quantitatively, on cv. Shiraz, the vines treated showed, on average in the three vintages, an increase of 8% in total anthocyanins, 10% in extractability of anthocyanins, and 29% in concentration of tannins. In all the treatments performed, the sensorial analysis of the wines showed a preference for those from treated vines. Especially in the case of Sauvignon blanc, these preferences were due to greater aromatic intensity and fresh and fruity wines. The study showed, with no significant changes in agronomic parameters, a positive impact of the treatment on the sensory quality of wines produced.

Funding Support: Universidad Politécnica de Madrid

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Depolymerization of Proanthocyanidins from Grape Seed Extract

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As flavan-3-ol monomers are better absorbed than the polymer, there is interest in a treatment of the polymerized proanthocyanidins in grape seed extract (GSE) that would reduce the average degree of polymerization, specifically enhancing the amount of monomer and dimer. Attempts to mimic thiolysis with a hydride nucleophile using an acid stable borohydride were not successful. However, previous work demonstrated that acidic conditions to cleave the interflavan bond in proanthocyanidins could be combined with SO₂ to provide nucleophilic addition of -SO₃H (sulfonates). Sulfur dioxide and ascorbic acid were combined with GSE in an ethanolic GSE solution. High-performance liquid chromatography (C18 R-HPLC) coupled with electrospray ionization mass spectrometry was used to identify and quantify the monomers through trimers. Procyanidin separation by degree of polymerization was performed using a diol column. Surprisingly, the reaction increased monomers and shorter polymers, with only partial sulfonic acid substitution on the compounds that were “depolymerized,” largely leading to direct depolymerization. The reaction was pH and temperature dependent. Although the exact mechanism is unknown, it appears that several reactions are occurring. One, as expected, the sulfite nucleophile is participating in an addition reaction at the carbocation position as sulfonate addition products are observed. Two, most of the product arises from a direct reduction (perhaps analogous to quinone reduction with sulfite or ascorbate). The treatment of GSE with sulfite and ascorbate in the presence of strong acid significantly increases the amount of monomer and dimer, while producing sulfonates as a byproduct of the reaction.

Funding Support: Polyphenolics, Inc.

Evaluation of Pigmented Tannin Structural Characteristics by a Complementary Suite of Mass Spectrometric Techniques

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In addition to providing the molecular basis for persistent red wine color, pigmented tannin has been shown to alter flavor perception and astringency and to cause softening of texture and mouthfeel. Pigmented tannin comprises a significant portion of the red wine matrix, and while some components have been identified, an inventory of compounds that constitute pigmented tannin remains fragmentary, despite its importance. To begin the identification, we are using Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry,

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which provides high-accuracy molecular weights for mixtures of numerous compounds. These precise mass values lead to almost unique molecular formulae, while less precision would leave dozens, if not hundreds, of options. These formulae are then paired with published structures where known and, to date, approximately 150 have been matched. To address the unknowns, we have developed a customized diol phase nano-HPLC chromatographic method for utilization with tandem quadrupole time of flight (QTOF) mass spectrometry. This will allow fragmentation analysis to create information on molecular connectivity necessary to properly determine structure from the FTICR molecular formula. To date, we have found over 100 unknown compound candidate ions, and the determination of these structures may confirm proposed structures and bring to light new structures for which the mechanisms of production are unknown. Knowledge of pigmented tannin structure provides the basis for understanding their production mechanisms, which in turn will lead to cellar control for winemakers looking to enhance or suppress the formation of specific wine pigments.

Funding Support: American Vineyard Foundation

Effect of Exogenous Acetaldehyde during Fermentation on Red Wine Tannins and Astringency

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Tannin profiles of red wines are known to affect the sensorial quality of the wine where a balanced astringency is desired. It is accepted that the sensation of astringency is due to an interaction between tannins and salivary proteins wherein their aggregation leads to precipitation and subsequent loss of lubrication in the mouth. Tannins undergo modifications during fermentation and storage that alter their concentration and/or structures and can decrease their perceived astringency. While many oxidation reactions in red wine are known to occur, the link between these reaction products and resulting astringency is not fully understood. Acetaldehyde is known to act as a bridging compound to form modified tannins and polymeric pigments that are less likely to form precipitable tannin-protein complexes than unmodified tannins. Here, we look at the effect of exogenous acetaldehyde at low and high treatment concentrations added during the primary fermentation of an unsulfited red wine. We hypothesize that these acetaldehyde treatments will lead to an increase in polymeric pigments and a decrease in astringency as measured by salivary protein precipitation. Our results show that high acetaldehyde treatment (1000 mg/L in the must throughout fermentation) significantly increased polymeric pigments in the wine without increasing concentrations of free and sulfite-bound acetaldehyde. This treatment also resulted in a decrease of total phenolics and an increase in the chemical age of the wine. The effects on astringency were

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also assessed. These results demonstrate the feasibility of acetaldehyde treatment early in red wine production as a means to increase color stability and modify the tannins of the finished wine.

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Survey of Phenolics in North Carolina Wines

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To assess the phenolic composition of North Carolina wines, 169 samples of commercial red wine were collected at the North Carolina State Fair Wine Competition in Oct 2012. At least 75% of the grapes used for vinification were grown in North Carolina. The 75% composition was also implemented for inclusion of cultivars and species in their respective categories. All wines were analyzed using the Adams–Harbertson assay. Mean anthocyanin concentrations in Chambourcin and Noble wines were higher than mean anthocyanin concentrations in all *Vitis vinifera* wines. Chambourcin and Cabernet franc had the highest and Sangiovese the lowest small polymeric pigment content of the cultivars tested. Tannin concentrations of 72% of *V. vinifera* wines were ≤ 450 mg/L CE; 71% of the French-American hybrid wines were ≤ 300 mg/L CE; and only one muscadine wine had a concentration ≥ 250 mg/L CE. Almost a four-fold difference in anthocyanin concentration was found due to vintage between the lowest and highest concentrations. Our data support the observation that North Carolina *V. vinifera* wines are likely to be perceived as less astringent than wines from Washington and California based on tannin concentration and are low in anthocyanin concentration, hence relatively low in red color.

Funding Support: North Carolina Department of Agriculture, North Carolina State University

Limits on Red Wine Tannin Extraction and Additions: The Role of Pathogenesis-related Proteins

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It has been previously documented that wines produced from disease resistant, cold-hardy, interspecific hybrid grapes have a “thin” mouthfeel, resulting from low wine tannin. Furthermore, adding exogenous tannin to fermentations does not consistently mitigate this low tannin issue. To investigate this phenomena, three wild *Vitis* species (*Vitis aestivalis*, *V. cinerea*, and *V. riparia*), four interspecific hybrid (Baco noir, Chancellor, DeChaunac, and Maréchal Foch), and

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four *V. vinifera* (Cabernet franc, Cabernet Sauvignon, Merlot, and Pinot noir) cultivars were harvested in the Finger Lakes region of New York and fermented in triplicate under similar conditions. Purified soluble grape seed tannin was added to each wine at an addition rate of 150 mg/L and allowed to incubate for 30 min, followed by a centrifugation step. Pelleted tannin from wine was then resuspended and measured via reaction with ferric chloride. In the *Vitis* (spp.) wines, an average of 74% of the added tannin was recovered in the pellet, followed by interspecific hybrid wines (56%) and *V. vinifera* wines (34%). The pellets also contained protein, which were determined by SDS-PAGE separation and proteomic analysis to be VVTL1 precursor, class IV chitinase precursor, and β -1,3-glucanase. These pathogenesis-related proteins (PRPs) have been studied extensively in white wine haze formation, but the tannin-binding role of PRPs in red hybrid and wild *Vitis* (spp.) red wines is not previously reported. We hypothesize that the amount of PRPs in interspecific hybrid wines is intrinsically higher due to their selection for disease resistance and can account for variation in wine tannin documented between select wine cultivars.

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

Merlot Versus Cabernet Sauvignon Brand and Consumer Selection

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The United States Department of Commerce estimated that in 2010, California accounted for 61% of all wines sold in the U.S. market, imported wines accounted for 31%, and wines from the other 49 states accounted for 8%. Given the wide variety of wine, this research project focused on the two most sold red wines in the United States: Merlot and Cabernet Sauvignon. Cabernet Sauvignon is the most popular red winegrape varietal consumed and purchased in the United States. The grape varietal Merlot is the second most sold red wine in the United States. This original marketing research study investigated what influences a consumer to purchase one brand versus another brand of Merlot or Cabernet Sauvignon from retailers. When buying Merlot or Cabernet Sauvignon wine, participants (n = 172) in the study would purchase wine based on a minimum of two criteria. Statistical results found that Merlot consumers were more likely to be male, younger, and to report lower income, whereas Cabernet Sauvignon consumers were more likely to be female, older, and to report higher income. The study statistically analyzed labeling, screwtop versus cork, price, promotion, word-of-mouth advertising, awards, and taste ratings as variables that may influence consumer selection.

Funding Support: Self-funded California State University, East Bay MBA study

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Effect of Wine-based Dosage Solutions on the Volatile Aroma Composition and Flavor of Ontario Sparkling Wine

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The aim of this research was to investigate chemical and sensory effects of various wine-based dosage solutions, including zero-dosage, on Ontario sparkling wine aroma and flavor quality. No studies exist that have investigated the effect of sugar addition, sugar type, or oak-derived volatile aroma compounds on sparkling wines post-disgorging and during storage. Trial treatments included: current release wine (zero-dosage); current release wine + sugar; unoaked still Chardonnay wine + sugar; oaked still Chardonnay wine + sugar; Blanc de noirs 2009 sparkling wine + sugar; Niagara produced brandy + sugar; and icewine (with no added sugar). TA (g/L), pH, VA (g/L) alcohol, free and total SO₂, and residual sugar levels were analyzed prior to and post-addition of dosage solutions to ensure residual sugar level of 8 g/L in all treatments. Brut sparkling wine, a blend of Chardonnay and Pinot noir wines, with 12 months lees aging, was used for the trial. Volatile aroma composition was carried out on wines prior to dosage, post-dosage, and on the actual dosage solutions by HS-SPME-GC-MS-SIDA at 5 weeks and 10 weeks post-disgorging. Results were analyzed using ANOVA and principal component analysis by XLSTAT.

Funding Support: Trius Winery, Peller Estates, CCOVI Brock University

Postharvest Impact of Brown Marmorated Stink Bug (*Halyomorpha halys*) Taint in Wine

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Agriculture crops across the United States are threatened by invasive pests. Much work focuses on preharvest control, but for wine, postharvest impacts are of particular importance. For brown marmorated stink bug (BMSB), current pest management techniques are limited. Consequently, this insect is found in the cluster during harvest and therefore is present during grape processing. *trans*-2-Decenal is the main taint compound associated with BMSB due to its strong odor, described as musty or green. The objective of this study was to understand the effect of wine processing on *trans*-2-decenal concentrations. A relationship between the BMSB density in grapes with the consumer rejection threshold of *trans*-2-decenal (4.8 µg/L) has been determined. The impact of different BMSB densities in grapes with several wine-processing steps was investigated. The bug density levels were: (a) control (no bugs); (b)

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one bug per three clusters (T1); (c) one bug per cluster (T2); and (d) three bugs per cluster (T3). The different processing steps investigated were type of press, pressure during press, and agitation prior to destemming (as a mimic of mechanical harvesting). Taint concentrations were measured using MDGC. The results indicate that the control was free of *trans*-2-decenal. Processes with the most impact included destemming and pressing. The concentration of taint compound decreased after destemming, possibly due to the high chemical reactivity of *trans*-2-decenal. The increased chemical stability of red wines after pressing caused taint levels to closely match their levels in the finished wine. Type of press and pressure program also affected the taint levels released during pressing. Therefore, pressing is considered as the critical step for BMSB taint in wine. The knowledge of the relationship between *trans*-2-decenal production and the parameters of wine processing allows winemakers to control *trans*-2-decenal levels in the finished wine and devise quality-control measures for wine processing.

Funding Support: Oregon Wine Research Institute

Impact of an Aroma-Enhancing Ester on Aroma Perception of a Riesling Wine Model

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Direct and indirect effects of *trans*-ethyl cinnamate (ethyl 3-phenyl-2-propenoate) were investigated using three different wine models: water-ethanol solution, dearomatized wine, and dearomatized wine supplemented with typical Riesling aroma matrix. Water-ethanol solution contained 10% v/v ethanol, 5 g/L tartaric acid, and pH was corrected to 3.2. Dearomatized wine was white wine that had been stripped of aroma compounds with liquid-liquid extraction. Sensory analyses were used to determine the olfactory detection threshold of the *trans*-ethyl cinnamate for the different wine models. Results showed that synergistic indirect effect exists between *trans*-ethyl cinnamate and typical Riesling wine matrix to aroma perception. Supplementing the samples with *trans*-ethyl cinnamate under its detection level lowered the detection threshold of Riesling matrix to half. The olfactory thresholds were for *trans*-ethyl cinnamate in water-ethanol solution 2.7 µg/L, dearomatized wine 8.55 µg/L and in typical Riesling aroma matrix 4.71 µg/L. The effect of dearomatization of the wine was confirmed with GC-MS.

Funding Support: Hochschule Geisenheim University

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Descriptive Analysis and Consumer Study of Viognier Wines from Virginia, France, and California

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A trained panel (n = 12) identified the key characteristics of Viognier wines from Virginia, France, and California. Fifteen aroma attributes were identified and assessed: artificial fruit, chemical, citrus, earthy/dusty/musty, floral, green apple, hay, honey, hot (ethanol), melon/cucumber, stone fruit, sulfidic, tropical fruit, woody, and yeasty. Six taste and mouthfeel attributes were measured: astringent, bitter, hot (ethanol), sour, sweet, and viscosity. In addition to the aroma and taste attributes, overall product intensity was assessed. Principal component analysis and analysis of variance were used to describe and differentiate among wines and countries. Overall product intensity, artificial fruit, honey, hot, stone fruit, sulfidic, tropical fruit, viscosity, sweet, sour, astringent, and hot mouthfeel were all found to be significant across wines. When analyzing product versus region, wines from California were found to be significantly greater in overall intensity, stone fruit, artificial fruit, and tropical fruit than wines from Virginia and France. French Viognier wines were found to be significantly greater in woody than Virginia or California Viognier wines. Consumer studies in Virginia (n = 193) and California (n = 109) found a preference for wines high in fruit intensities and with higher residual sugar and acidity.

Funding Support: Virginia Wine Board

Plastic Polymers in the Wine Industry: HACCP Considerations

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Plastics are ubiquitous among food and beverage packaging materials and their use is expanding, including packaging materials for wines. Plasticizers are a class of synthetic organic compounds used to modify and improve the applicability and durability of plastics. Common plasticizers include phthalates and bisphenol A (BPA). Phthalates are a group of ortho-phthalic acid diesters (dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid) typically used to soften plastics. BPA belongs to a group of diphenylmethane derivatives used to harden plastics. Phthalates are used in many products from polyvinyl chloride (PVC), lubricants, emulsifying agents, binders and gelling agents, to enteric coatings on pharmaceutical tablets. BPA is commonly used in polycarbonate plastics and epoxy coatings. As plasticizers, phthalates do not chemically bind to the plastics in which they are mixed, and plastic-BPA polymers can be hy-

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dolyzed; therefore, both types of plasticizers can leach from the products into the environment. Plasticizers are in widespread use and are an environmental and human health concern. Contamination of foodstuffs such as wine with plasticizers can occur through food-contact processing and packaging materials and during processing, storage, and transport. The ubiquitous use of plasticizers in general and the increasing industry use of plastics in wine storage and packaging have led to detection of plasticizers in wines and in extracts from plastic stoppers and corks. GC-MS and LC-MS are among the most common analytical tools to analyze for plasticizers. This work reviews plastics use in the wine industry, environmental and animal health concerns with plasticizers, and current regulations regarding plasticizers that may be relevant to the wine industry and academic and scientific wine endeavors.

Funding Support: programatic support

Relationship of Yeast Dynamics and Chemical/Sensory Profiles of Inoculated and Spontaneous Chardonnay and Pinot noir

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The Okanagan Valley has become a valuable wine region in western Canada, growing rapidly and rivaling other wine regions in both wine quality and varieties grown. As competition increases, winemakers seek to improve and distinguish their product to produce unique, distinguishable wines. Despite spoilage risks, wineries may seek to create unique wines by including spontaneous fermentations in the final blend, allowing fermentation by yeast flora already present on the grape surfaces and in the winery. Spontaneous and inoculated fermentations of Pinot noir tanks and Chardonnay barrels at Quails' Gate Estate Winery were monitored over the 2013 vintage. Following colony isolation and microsatellite analysis of yeasts present at four fermentation stages, it appeared that the addition of inoculum did little to influence the yeast community in inoculated Pinot noir. Aggressive *Saccharomyces cerevisiae* strains used previously by the winery were responsible for the majority of Pinot noir fermentations. Although spontaneous Chardonnay barrels were not inoculated, the inoculum used in this vintage was equally dominant throughout both inoculated and spontaneous fermentations. Interestingly, it was found that the population of non-*Saccharomyces* species was prevalent from early to final fermentation for both varietals, adding to data that suggests non-*Saccharomyces* species persist in and contribute to the fermentation of wines. To understand the influence of different yeast populations present in each tank and barrel on the chemical profile of the wines, samples collected immediately following fermentation and prior to malolactic fermentation will be analyzed through gas chromatography and

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compared for any similarities and significant chemical differences. Subsequently, a sensory analysis by a panel of experts will be conducted for all fermentations from both varieties. The results of both chemical profiles and sensory analysis will be reported.

Funding Support: NSERC, Quails' Gate Estate Winery

Determining the Origin of Fermentation Volatiles Using Labeled Glucose Tracers and High-Precision Isotope Ratio Analyses

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Many key wine odorants can arise both from yeast metabolism of sugars or through abiotic and biotic transformations of other grape-derived compounds, but it is often not evident which pathway is dominant. For example, the fusel alcohols and their corresponding acetate esters can be formed by enzymatic degradation of grape amino acids as well as by *de novo* biosynthesis from hexose sugars. Potentially, tracer experiments using stable isotope-labeled hexose substrates could be used to resolve questions regarding the relative importance of pathways, but tracer experiments with sugars are challenging because of the high concentration of endogenous sugars in must. Here, we report using high-precision gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) to track the fate of tracer levels (0.01 to 1% atom excess) of uniformly labeled [U-¹³C] glucose during fermentation. The contribution of hexoses to a diverse range of fermentation volatiles, including fatty acids, ethyl esters, acetate esters, and higher alcohols, was calculated from the degree of ¹³C enrichment. As expected, fatty acids and their corresponding ethyl esters were >90% derived from hexoses, with the exception of the branched-chain isobutyric acid (50% hexose derived). Isoamyl alcohol and other fusel alcohols, as well as their corresponding acetate esters, were >75% derived from hexoses, indicating amino acid degradation is of only minor importance to forming these compounds. The only volatile under study that was not majority-derived from hexoses was hexanol (<10% hexose), likely because it arises primarily through grape lipid oxidation. The GC-C-IRMS approach described here could be extended to other labeled precursors such as amino acids and to study formation of nonvolatiles.

Funding Support: USDA–Hatch Funds

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Enology – CONTINUED

Quantifying Cumulative Wine Oxidation via Acetaldehyde Condensation Reaction Products

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At low levels, oxygen can play a significantly positive role in the development of a wine. High oxygen exposure, however, can lead to undesirable changes in its aroma and flavor. Consequently, methods for monitoring the degree to which wines have been exposed to oxygen are essential for gaining control of the oxidative changes in wines. The significant production of acetaldehyde via oxidation of ethanol by the hydroxyl radical has been taken into consideration in previous studies, prompting the measurement of acetaldehyde, in addition to other aldehydes present in wine, to determine oxygen exposure and aging. However, acetaldehyde is a poor choice for measuring oxidation, as it is still very reactive. Its concentration in wine varies widely due to its interaction with other wine components such as anthocyanins, flavanols, and alcohols. Due to the complexity and polymerization of dimers and oligomers yielded by some of these interactions, the most promising, stable reaction products of acetaldehyde are acetals. Formed by the condensation of acetaldehyde with wine alcohols, acetals have shown good correlation with age in fortified wines. The analysis of important acetals from acetaldehyde and glycerol in wine by liquid-liquid extraction followed by gas chromatography mass spectrometry has been successfully optimized to improve recovery and reduce sample preparation time. The rate of formation and hydrolysis of heterocyclic acetal alcohols was measured in model wine solutions. Data was used to calculate rate constants for the forward and reverse reactions, as well as the equilibrium constant under wine-like conditions. Comparison of the chemical kinetics of these reactions with others occurring in wine will be conducted by competitive experiments, allowing for the determination of the fraction of acetaldehyde in solution that reacts to give acetals. This data will aid in quantifying oxygen consumption in wine by the measurement of acetal content in wines.

Funding Support: American Viticulture Foundation

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Enology – CONTINUED

Pectinase-Catalyzed Esterification of Cinnamic Acids with Ethanol during Winemaking

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Dry white wine made from Koshu (*Vitis vinifera* cv.) often has a bitter after-taste. Extremely high concentrations of ethyl *p*-coumarate and ethyl caffeate have been detected in some Koshu wines. It was previously reported that ethyl *p*-coumarate and ethyl caffeate contribute to the bitter taste of wine. Thus, those ethyl esters might contribute to the bitterness of Koshu wines. Because those compounds were detected in Koshu wine but not Koshu grape juice, it is speculated that they are formed during winemaking. Some commercially available enzyme preparations (pectinase) have been shown to catalyze the esterification of ferulic acid. Therefore, ethyl *p*-coumarate and ethyl caffeate also may be formed from *p*-coumaric and caffeic acids such enzyme preparations, and those cinnamic acids may be formed from caftaric acid (tartaric ester of caffeic acid) and coutaric acid (tartaric ester of *p*-coumaric acid) via the same esterase activity. In this study, we investigated the reactions of cinnamic acids with enzyme preparations in buffer solution and under experimental winemaking conditions. Among the 10 enzyme preparations investigated, two showed esterification and de-esterification activities. The two enzyme preparations were added (200 mg/L) to Koshu grape juice (containing 83 mg/L coutaric acid, 230 mg/L caftaric acid, 0.3 mg/L *p*-coumaric acid, and 0.3 mg/L caffeic acid) and fermentation was induced with EC-1118. Coutaric and caftaric acids were almost completely hydrolyzed to *p*-coumaric and caffeic acids within 12 days. Ethyl *p*-coumarate and ethyl caffeate were formed as the amount of ethanol was increased. Our findings suggest that some pectinases hydrolyze coutaric and caftaric acids to *p*-coumaric acid and caffeic acid and those cinnamic acids are esterified with ethanol formed during the fermentation process.

Funding Support: Grant-in-Aid for Young Scientists B

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Enology – CONTINUED

Effect of Cofermentation of Red Grapes on Tannin Extraction and Composition

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The cofermentation of red grapes is a winemaking technique that is used by some winemakers. In this process, two or more grape varieties are cofermented; in contrast to the process where varieties are fermented individually and then blended following fermentation. It is generally considered by practitioners of cofermentation that fermenting different varieties together improves the perception of specific wine attributes, including mouthfeel. The purpose of this study was to investigate the role of cofermentation on the extraction and composition of tannins in red grapes, with particular attention being given to tannins. Harvested fruit from coplanted grapes (*Vitis vinifera* L. cvs. Alicante Bouschet, Carignane, Mataro, Petite Sirah, Zinfandel) were sourced from the Dry Creek Valley located in Sonoma County, California (38°39'14.93"N, 122°53'30.99"W) and were fermented individually or together (based upon proportions present in the vineyard) in research-scale 4-kg fermenters (three replicates). Grape and wine tannins were analyzed by phloroglucinolysis which provided information on tannin concentration, composition, and mean degree of polymerization. In addition, thermodynamics of wine tannin interaction with a hydrophobic surface was determined. Based upon berry analyses of skin and seed tissue extracts prepared from individual varieties, significant variation in tannin concentration, composition, and degree of polymerization existed. However, variation in grape tannin chemistry across variety was inconsistent with variation in wine tannin chemistry across variety. Differences in tannin chemistry between single-variety wines and the corresponding cofermented wine suggest that cofermenting grapes has some impact on chemistry beyond expectation. Taken together, these results suggest that the tannin chemistry of blended wines cannot replicate the tannin chemistry of wines when grapes are cofermented.

Funding Support: American Vineyard Foundation Ridge Vineyards, Inc

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Viticulture

Effect of Early Lag-Phase Thinning on Low-Vigor Zinfandel

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A consistently underperforming block of Zinfandel with leafroll viral symptoms was selected for study. The block, located in Dry Creek Valley in Sonoma County, California (38°39'14.93"N, 122°53'30.99"W), underwent a thinning experiment in 2013. The crop on treatment vines was early lag-phase thinned by 20% (2–3 clusters per vine) beginning ~45 days after 50% bloom (treatment). Control vines were left unthinned, until 95% veraison, at which point a green drop pass was made (control). Berry samples from treatment vines showed more rapid progress through veraison, an increased rate in sugar accumulation and were ready for harvest three weeks earlier than control vines. Wines were produced in research-scale 4 kg fermenters (three replicates). Grape and wine tannins were analyzed by phloroglucinolysis, which provided information on tannin concentration, composition and mean degree of polymerization. In addition, the thermodynamics of wine tannin interaction with a hydrophobic surface was determined. Wines produced from treatment vines showed not only a higher quantity of extractable tannins but tannins with a more exothermic enthalpy of interaction with a hydrophobic surface. Phloroglucinolysis information indicated that although the skin tissue of the control berries contained significantly more tannin on a per berry level and with a higher mean degree of polymerization, the tannin was not extractable during fermentation. In addition to an increase in skin tannin amount for treatment wines, they also contained proportionally more skin tannin (10% increase), indicating that the timing of the thinning pass significantly affected skin tannin extractability. In addition, commercial fermentations of the treatment parcels appear to be of the highest quality yet from the block under study.

Funding Support: American Vineyard Foundation, Ridge Vineyards

Identification of True Hybrids of *Vitis aestivalis*-derived Norton-based Populations Using Molecular Markers

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Vitis aestivalis-derived Norton is a commercial red winegrape variety that can be grown in regions with high disease pressure and cold winter temperatures where *Vitis vinifera* is difficult to grow. The unique characteristics of fungal disease resistance in Norton grape render it an ideal candidate to generate interspecific

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hybrids with improved viticultural performance and enological quality. This study initiated crossing Norton with *V. vinifera* varieties Cabernet Sauvignon, Syrah, Pinot noir, and Merlot and the *Vitis* interspecific hybrid Vignoles. The true hybrids were identified using microsatellite markers and single nucleotide polymorphism (SNP) markers generated from genotyping-by-sequencing (GBS). A high percentage of hybrids were acquired in all crosses (70 to 93%) except for Norton x Merlot (16.6%), where all the nonhybrids showed the Norton microsatellite banding patterns, indicating a probable emasculation error resulting in self-pollinated plants. This work emphasizes the efficiency of molecular markers that can be used to greatly accelerate the breeding process and allow a much more accurate selection of progeny.

Funding Support: Missouri Department of Agriculture Specialty Crop Block Grant

Population Dynamics, Gene Flow, and Sources of Chloride Exclusion in Wild *Vitis* from the Southwest United States

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Most commercial grapevine rootstocks are derived from three North American *Vitis* species (*V. rupestris*, *V. berlandieri*, *V. riparia*) and were developed mainly based on resistance to phylloxera, ease of propagation, and tolerance to calcareous soils. However, the focus of rootstock breeding is now shifting toward an expanded suite of characteristics, including salt tolerance in the form of chloride exclusion and utilization of more genetically diverse plant material. Previously, we screened a subset of our grape germplasm collection for chloride exclusion and found several promising sources from wild *Vitis* populations collected in the southwestern United States. All wild *Vitis* species are dioecious (separate male and female plants) and easily form interspecific hybrids where their ranges overlap. This presents a complex problem for systematics, especially in the southwestern United States where several species' ranges meet in a largely fragmented habitat. In this study, we pair the results of chloride exclusion screens for our entire collection of southwestern U.S. *Vitis* with a large-scale population genetics analysis based on genotyping with nuclear and chloroplast microsatellite markers. We will present the results of this analysis in light of identifying multiple distinct genetic sources of chloride exclusion for rootstock breeding, as well as gaining a greater understanding of the overall population structure and patterns of gene flow in wild *Vitis* populations. A clearer picture of the population dynamics and taxonomy of these vines is crucial for efficient rootstock breeding and for future conservation efforts.

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

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Viticulture – CONTINUED

Relationship between the Formation of Upper Lateral Sinus and *VvNAC21/22-like* Expression in Grapevine Leaves

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Grapevine leaf has three unique sinuses. We report here the relationship between the formation of the upper lateral sinus and the expression of *VvNAC21/22-like*, a member of grape NAC transcription factor family. *VvNAC21/22-like* expression was not upregulated in young leaves of all cultivars tested and in mature leaves of cultivars showing shallow sinuses, whereas mature leaves of cultivars showing deep sinuses expressed *VvNAC21/22-like* abundantly. Simple linear regression analysis demonstrated that *VvNAC21/22-like* expression, but not other NAC proteins, *VvNAC1*, *VvCUC2*, and *VvCUC3*, in mature leaves showed strong positive correlation with the depth of upper lateral sinus in mature leaves, suggesting that the increase in *VvNAC21/22-like* expression at the late stage of leaf maturation was related to the formation of deep upper lateral sinus in grapevine leaves. Distribution of *VvNAC21/22-like* mRNAs in grapevine leaves was found to be the upper and lower lateral sinuses. From the above correlation study, we hypothesize that *VvNAC21/22-like* may play a role in the formation of the upper lateral sinus on grapevine leaves. Our findings are expected to offer the possibility of sinuses on grapevine leaf as a genetically breeding target for adaptation to environmental conditions.

Funding Support: University of Yamanashi

Are Norton and Cynthiana Synonyms? A Genome-wide Comparative Assessment Using Microsatellite Markers

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Norton and Cynthiana grape cultivars are both described to be largely derived from *Vitis aestivalis*. Although isozyme analysis in 1993 provided preliminary evidence that these two cultivars were genetically identical, only five banding patterns were reported. Despite these data, Norton has been described in numerous publications as distinct from Cynthiana, and growers and winemakers assert that distinctions exist in their respective viticultural performance and enological quality. We are currently characterizing the relationship between these two cultivars using microsatellites, a simple and efficient procedure for genome-wide analysis. Microsatellites, or simple sequence repeats (SSRs), are one of the most popular sources of genetic markers and play a significant role

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in plant genetics and breeding. They present the advantage of being PCR-derived, codominant, highly polymorphic and have proved their usefulness for the genetic analysis of a heterozygous species-like grape. In concert with this study, we constructed a genetic map of a *V. aestivalis*-derived Norton and *V. vinifera* Cabernet Sauvignon population by testing 600 SSR markers, 359 of which were informative markers polymorphic for Norton in 19 chromosomes. Leaf DNA will be isolated from three accessions of Cynthiana and four accessions of Norton in Arkansas and Missouri. Ten randomly chosen SSR markers from each chromosome will be used to compare the microsatellite banding patterns between Norton and Cynthiana. Genotype assessment at these SSR loci will be compared, contrasted, and discussed.

Funding Support: Missouri State University Faculty Research Grant

Genetic Mapping of Powdery Mildew Resistance in the Wild Chinese Species *Vitis piasezkii*

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The fungus that causes grapevine powdery mildew, *Erysiphe necator*, is an obligate ascomycete that attacks members of the grape family. The disease it causes damages leaves, impacts vine growth, and is capable of destroying the crop. To prevent mildew disease, fungicides are applied every 7 to 21 days during the growing season. Breeding efforts to control this disease commenced 150 years ago. American *Vitis* species have been the traditional source of resistance, but more recently resistance has been discovered in Asiatic *Vitis*. These species are easily crossed with other *V. vinifera* varieties, and the fruit quality of the resulting progeny is generally superior to crosses made with North American *Vitis* species. This study presents a SSR marker-based framework linkage map of the susceptible *V. vinifera* F2-35 x *V. piasezkii* (DVIT 2027) a highly resistant Chinese species. The 288 progeny have been tested for mildew resistance in the field and greenhouse and with two different in vitro assays. Over 500 SSR markers were screened and 200 polymorphic markers were added to the entire population. The genetic map was developed using JOINMAP software and QTL analysis was carried out using interval mapping and composite interval mapping. Major disease resistance loci were located on chromosome 9 and 19, which are novel locations compared to previous powdery mildew mapping efforts. These alternative resistance loci will aid grape breeders as they attempt to stack resistance genes to ensure durable resistance.

Funding Support: Corvinus University of Budapest and the American Vineyard Foundation

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In Vitro Induction of Tetraploids in *Muscadina* Hybrid Rootstocks

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Muscadina rotundifolia is naturally resistant to most grapevine diseases and pests. However, differences in chromosome number and consequent sterile hybrids with *Vitis* spp. pose a major obstacle for its use in rootstock breeding. The objective of this study was to develop a protocol for producing fertile tetraploid hybrids between 101-14 Mgt ($2n = 2X = 38$) and *M. rotundifolia* cv. Trayshed ($2n = 2X = 40$). Different explant materials were exposed to three concentrations of two antimitotic chemical agents, colchicine and oryzalin for 24, 48, and 72 hr. Treated shoot tips, anthers, and embryos were grown into plants and ploidy level was evaluated by chromosome counts and flow cytometry. Morphological and anatomical characteristics of leaves, including leaf index, stomata size, and number of chloroplasts in guard cells were also analyzed. Results showed that leaf characteristics correlated with ploidy of grape plants. The number of chloroplasts in guard cell of stomata resulted in a stable and reliable marker and was therefore used in subsequent screenings. Tetraploid plants were then confirmed by chromosome counts and flow cytometry. A relatively simple method for obtaining a high frequency of *Muscadina* hybrid tetraploids based on treatment of shoot tips with colchicine will be presented and discussed in light of the different variables involved in the process.

Funding Support: China Scholarship Council, Louis P. Martini Endowed Chair, California Grape Rootstock Improvement Commission

Trellis Conversion of Traditional Systems and Irrigation Stress Effects on a Procumbent Grapevine in Warm Climate

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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 under irrigation stress on vegetative compensation, yield components, whole canopy photosynthesis, and phenolic composition of fruit and wine of Merlot/Freedom grapevine. The following factors, pruning systems, and irrigation stress were arranged factorially in three randomized complete blocks. Pruning systems included a spur-spur-pruned CS trellis (HP), a cane-pruned CS trellis (CP), and a mechanically pruned single high-wire trellis (SHMP). Irrigation treatments were sustained deficit irrigation, where vines were maintained at a midday leaf water potential

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(Ψ) of -1.2 MPa and were irrigated to 80% of evapotranspiration (ET_0) from budbreak until harvest, and regulated deficit irrigation (RDI) that received 80% of ET_0 from budbreak to fruit set, whereafter 50% ET_0 was replaced to maintain (Ψ) at -1.4 MPa until véraison, but not thereafter. The leaf layer of CP was 17% and 14% higher than that of SHMP and HP, respectively. The yields of HP and CP 28% higher compared to SHMP. CP produced grapes with highest Brix and pH with lowest acidity. Pruning systems did not affect pruning weight but the crop load of SHMP was 34% and 41% lower than that of CP and HP, respectively. The phenolics measured included anthocyanins, proanthocyanidins, flavonols, flavan-3-ols, and nonflavonoid compounds. The concentration of caffeic acid was 9% higher in CP and SHMP compared to HP. Irrigation did not affect the canopy architecture, yield components, fruit composition, yield efficiency, and phenolic compositions in wine. This project provides applied information about the optimum crop load management and deficit irrigation strategies for maintaining or increasing yield and phenolic concentrations during the transition between manual and mechanical management vineyards.

Funding Support: American Vineyard Foundation

Interactive Effect of Crop Load and Skin Contact Time on the Phenolic Composition of Malbec Wines from Argentina

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The aim of this study was to evaluate the incidence of maceration length on the phenolic composition of Malbec wines from vines with different shoot number. The assay was performed in the 2013 season in a commercial vineyard located in Mendoza, Argentina. The grapevines were trained on a VSP system, and spur-pruned on a bilateral cordon. Field treatments corresponded to three different shoot numbers per plant (R1, 38; R2, 22; and R3, 16 shoots/plant, respectively). The experiment was installed in a complete randomized block design with five replicates. To study the interaction between field treatments and skin contact time, the grapes of each replicate were harvested at 24 to 25 Brix and elaborated by applying a standard protocol with different maceration length (M1, 7; M2, 14; and M3, 21-day skin contact, respectively). Global phenolic composition was studied by spectrophotometric techniques, and individual phenolics were evaluated by HPLC-DAD/ESI-MS, in wines bottled at 45 days after racking. Our results showed that shoot number imparted a greater effect on most chemical compounds compared with maceration length.

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We did not observe any interaction between field and maceration treatments. We found a significant increase of global phenolics in wines from R3 plants. These plants allowed producing wines with the highest color intensity, and the highest levels of pyranoanthocyanins, anthocyanin-flavanol adducts, phenolic acids, and flavonols. In contrast, maceration length only affected tannin content, with 25% more in M3 than in M1. M2 wines showed a higher concentration of monomeric anthocyanins, polymeric pigments, and total phenols. We demonstrated that field treatment played a main role on wine composition and that extended skin contact may not overcome the effects of poor berry quality. The combination of R3 with M2 treatments allowed obtaining wines with great potential for aging due to color stabilization through copigmentation, condensation, and polymerization reactions.

Funding Support: Instituto Nacional de Tecnología Agropecuaria (INTA), projects PNAI, AV 1130043, and PNFRU-1105064

Evaluation of Canopy Management Practices for Norton Grapevines

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Norton is an economically important cultivar in the Midwest United States that produces high-quality wine while also having excellent disease resistance. However, growing Norton is a challenge due to low productivity combined with high vegetative growth, producing dense canopies. Norton is also challenging to vinify due to fruit with high potassium, pH, malic acid, and titratable acidity at harvest. Leaf removal alone has mixed results in improving problems with Norton, necessitating further investigation of the effects of using multiple canopy management techniques. The effects of shoot thinning, shoot positioning, and leaf removal, separately and in combination, were investigated at two Missouri sites from 2006 to 2008 (phase 1) and 2009 to 2010 (phase 2). The sites consisted of a low vine size vineyard in the southwest and a high vine size vineyard in central Missouri. Treatments were control, shoot thinning, shoot positioning, east side leaf removal, and all combinations of the latter three treatments. Phase 1 impacted vine size, vegetative growth, and yield for both sites; however, there were only minor impacts on fruit composition. These results initiated phase 2 to test if canopy asymmetry existed and if it was masking whole-vine response to canopy management treatments. Data were collected by vine side and point quadrat analysis was included. At both sites, shoot positioning reduced vine size, yield results varied, and canopy asymmetry occurred but did not influence fruit composition. At the high vine size site, canopy management treatments significantly affected fruit composition with full treatment, leaf removal, and shoot positioning plus leaf removal vines,

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while the low vine size site had minor but significant effects on fruit composition from full treatment vines. Overall, it was shown that canopy management practices can impact fruit composition of Norton grapes and reduce vine size although the extent of the impact is dependent on vine size and site.

Funding Support: Missouri Wine and Grape Board, Missouri Department of Agriculture Specialty Crop Block Grant

Conversion from Cordon Training and Spur Pruning to Head Training and Cane Pruning Yields Mixed Results

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Winegrowers have limited options to manage the often excessive vegetative growth of vigorous grapevines grown in humid regions. With a goal of reducing perennial wood retention, and possibly reducing vegetative vigor, some growers have adopted head training and cane pruning, as opposed to cordon training. We conducted an experiment with mature, cordon-trained vines to determine if conversion to head training and cane pruning was justifiable from the standpoint of vegetative growth suppression, maintenance of crop yield, and uniformity of crop ripeness. Seven year-old cordon-trained/spur-pruned (CT/SP) Cabernet Sauvignon (clone #337) grapevines were compared to similar vines that were converted to head training and cane pruning (cane pruned). Vine spacing was 2.7 m x 1.5 m. Cane-pruned vines produced 19% lower dormant cane-pruning weights than did CT/SP vines. Furthermore, canopies of cane-pruned vines had 20% fewer leaf layers than did CT/SP vines. Cane-pruned vines had 31% higher node fruitfulness (clusters/node) and fewer basal shoots emerged on these vines. Shoot length and caliper varied greatly between adjacent shoots on cane-pruned vines, while CT/SP vines displayed more uniform shoot growth and canopy uniformity. Total clusters per vine at harvest were higher for cane-pruned vines, but neither yield per vine nor cluster weight differed by training treatment. Primary fruit chemistry was similar between treatments based upon 50 berry samples collected from each treatment plot at harvest. Cane-pruned vines did have lighter prunings and less congested canopies than CT/SP vines, while crop yields remained similar. Budburst was delayed by up to 10 days for cane-pruned vines compared to CT/SP vines. While cane pruning reduced vine size and improved canopy characteristics, relative to CT/SP vines, the nonuniformity of the shoot growth along the canes remained a concern with respect to nonuniformity of crop ripeness.

Funding Support: Virginia Wine Board

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Delay of Budbreak on Edelweiss Grapevines with Multiple Applications of Amigo Oil and Naphthaleneacetic Acid

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Edelweiss is an important grape cultivar grown in the midwestern United States. It is one of the earliest cultivars in the vineyard to break bud, making it very susceptible to late spring freezes. Edelweiss primary buds produce a significant amount of fruit, while unlike many other hybrids the secondary and tertiary buds will have little to no yield, thus making it important to protect the primary buds from a late freeze. The objective of this research was to determine if multiple applications of Amigo Oil or naphthaleneacetic acid (NAA) achieve a greater bud delay than single applications. Edelweiss vines were treated in January, January and February, or January, February, and March. Amigo Oil was applied at 10% (v/v) and NAA at 1000 mg/L with a custom-built atv sprayer. All treatments of oil led to a significant budbreak delay ranging from 3 to 10 days as compared to the control. None of the treatments resulted in negative effects on yield or fruit characteristics. A controlled laboratory experiment was also conducted, where single bud cuttings were forced in 200 mg/L 8-hydroxyquinoline citrate and 2% sucrose at 25°C under 12-hr days. Treatments of one, two, and three applications of Amigo Oil and 1000 mg/L NAA were applied to single buds at weekly intervals. Julian days until budbreak were recorded and treatment-related budbreak delays were observed. Two and three applications of oil significantly delayed budbreak from 14 to 24 days. All NAA treatments led to significant bud delay ranging from 6 to 9 days. Grapegrowers in climates with the potential for late spring freezes may consider the use of Amigo Oil or NAA as a potential means to protect their vines from frost injury.

Funding Support: University of Nebraska Agricultural Research Division

Evaluating Canopy Leaf Area Sampling Protocols for VSP Canopies

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Quantification of grapevine canopy parameters is principal to understanding vine growth response to the environment and cultural practices. Nondestructive allometric methods to quantify leaf area have been developed based on the relationships between leaf area and other leaf parameters, mainly blade length and width. These methods are time intensive, requiring multiple linear measurements of numerous leaves. This study evaluated methods of quantifying canopy leaf area that may have greater accuracy and efficiency. Four commercial Oregon vineyards with various vigor levels were used for canopy leaf

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area measures of *Vitis vinifera* Pinot noir in 2013. Main shoot length, cumulative lateral shoot length, and individual leaf areas per shoot were quantified at véraison. Leaf areas were measured using a nondestructive field method where leaves were compared to a six category template. An index using shoot length multiplied by the average leaf size determined from the largest and smallest categorized leaves was used for statistical analysis. Measured leaf areas were compared to the index values and to calculated leaf area determined from the average of the largest and smallest categorized leaves multiplied by number of leaves. A positive linear relationship was established between the following: main shoot leaf area and indexed values, lateral shoot leaf area and index values, whole shoot leaf area and indexed values, main shoot leaf area and calculated leaf area, lateral shoot leaf area and calculated leaf area. A positive polynomial relationship was found between measured shoot leaf area and calculated shoot leaf area. Results indicate that calculated leaf area overestimated measured leaf area but did not differ for whole shoot and main shoot leaf area. The leaf area quantification methods used provide a more efficient alternative to destructive methods of whole vine leaf area in hedged VSP systems. Testing continues in 2014 to further develop models for enhanced accuracy.

Funding Support: Oregon Wine Research Institute and USDA-ARS

Yield Mapping in California Winegrape Vineyards

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Most California vineyards are developed and farmed uniformly, but spatial variation in topography and soil properties often results in within-vineyard variation of yield and fruit quality. Management of vineyard spatial variability is a main objective of precision viticulture, and geo-referenced mapping of vineyard attributes, including yield, is indispensable for this practice. Although yield maps are the cornerstone of precision farming in field crops, they have been absent in winegrape production in California. We report on the history, description, and use of yield monitors designed for mechanical grape harvesters and the commercial implementation of this system during the 2012 and 2013 harvests. Advanced Technology Viticulture yield monitors were successfully tested on two different mechanical grape harvester models during 2012 and 400 acres of vineyards were mapped at harvest. The resulting high-resolution yield maps were useful to understanding relationships among fruit quality, canopy vigor, and soil properties. Based on these results, yield maps were completed for 2,100 acres at harvest in 2013. Raw data processing, including cleaning of outliers and interpolation to final map, is discussed along with insights on decisions based on the resulting information.

Funding Support: E&J Gallo Winery

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Lateral Shoot Removal Increases Yeast-Assimilable Nitrogen

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The objective of this study was to determine the influence of amino-nitrogen (N) sources (leaves) and competing sinks (lateral shoots) on the loading of N assimilates into the fruit of Merlot grapevines grafted onto two rootstocks that differ in biomass production (vigor) in Oakville, California. In six consecutive vintages previous to the experiment, the fruit from Merlot vines grafted onto a low-medium vigor rootstock (101-14 Mgt) acquired approximately half the amount of yeast-assimilable nitrogen (YAN) than fruit from the same Merlot clone grafted onto a high-vigor rootstock (1103P). Consequently, the Merlot must from 101-14 Mgt rootstocks completed fermentation up to 8 days later than Merlot must from 1103P rootstocks and in some cases did not finish. In this study we tested the effects of reducing assimilate sources and sinks on YAN. Approximately half of the leaf biomass on 1103P rootstocks was targeted for early foliar reduction in attempt to reduce the canopy to equal that of 101-14 Mgt. Lateral shoots and mature leaves were removed from six 1103P vines except for nodes 15 and above, resulting in a 46% biomass reduction. The same was done with six 101-14 Mgt vines, resulting in a 12% biomass reduction. At harvest, juice YAN was measured. YAN from the altered vines increased equally between rootstock treatments compared to controls (85% for 101-14 Mgt and 91% for 1103P) despite the large differences in biomass reductions within and between rootstock treatments. The results suggest that sink competition for assimilates, i.e., demand by actively growing lateral shoots, influences amino-N loading into the fruit sink more than does source capacity, i.e., quantity of mature leaves producing assimilates. A principal finding of this investigation is that lateral shoot removal on winegrape cultivars grafted onto low-vigor rootstocks may lessen the incidence of slow or stuck fermentations.

Funding Support: American Vineyard Foundation

Understanding Vineyard Yield and Fruit Quality Variability

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Spatial variability in both yield and fruit quality in winegrape vineyards has been well described, although the primary causes for variability are poorly understood. The main purpose of this study was to apply precision viticulture tools to monitor and characterize fruit yield and quality covariation in a winegrape vineyard (*Vitis vinifera* Petite Sirah) in the Central Valley of California. Established protocol in 2013 was based on collection and mapping of high-

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density data for soil properties, canopy size and density, and fruit yield and quality attributes. Vine growth, productivity, vine nutrition, soil properties, and fruit chemistry were quantified for discrete data pods, selected to represent variability found in high-density canopy growth and yield maps constructed in the same block the previous year (2012). Statistical analyses of the data were designed to examine patterns of variability, quantify its magnitude, and study relationships among attributes. Mapping confirmed an inverse spatial relationship between fruit yield and quality variability in 2013. A magnitude of yield variability of five-fold (9.0 to 45.0 t/ha) was observed, while the difference in an index developed for fruit quality was only two-fold. Interannual variation in mean yields between 2012 and 2013 was low and was not persistent in spatial structure. Areas with the highest yield and lowest fruit quality coincided with greater potassium uptake at bloom and more count shoots per vine. These areas of the vineyard also corresponded to lower clay content and greater plant available water in the root zone. Fruit yield and quality covariation in the studied vineyard is best explained by natural variation in soil properties which contribute to the amount of available soil moisture. Site-specific management practices such as differential harvest or irrigation could therefore be considered useful to manage variability and optimize vineyard performance.

Funding Support: E&J Gallo Winery

Effects of Pruning Systems and Irrigation Stress on Yield and Phenolic Composition of Zinfandel in Hot Climate

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The objective of this study was to evaluate the effects of pruning systems and irrigation treatments on Zinfandel/Freedom. Pruning systems consisted of cane pruning (CP), hand pruning (HP), and mechanical pruning (MP). Irrigation treatments were sustained deficit irrigation (SDI) where vines were maintained at a midday leaf water potential (Ψ) of -1.2 MPa and were irrigated to 80% of evapotranspiration (ET_0) from budbreak until harvest, and regulated deficit irrigation (RDI) that received 80% of ET_0 from budbreak to fruit set, whereafter 50% ET_0 was replaced to maintain (Ψ) at -1.4 MPa until veraison, but not thereafter. Canopy microclimate, yield components, exhaustively extracted skin and seed, and partially extracted phenolics of grape must were measured. MP increased photosynthetically active radiation (PAR) compared CP and HP in fruit zone. SDI increased PAR by 16% compared to RDI. Leaf area of CP was 40% lower than HP and MP. RDI decreased berry size by 18% compared to SDI. CP had the highest yield compared to MP and HP. MP and CP had the highest Brix and pH, and HP and MP had the highest TA at harvest. The pH was 4% higher for RDI treatment compared to SDI and TA was 12% higher for SDI treatments compared to RDI. Pruning weights were 38% lower

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and Ravaz index was 49% higher for CP than HP and MP. Leaf area to fruit ratio was 110% higher for HP and MP than CP. The phenolics measured included anthocyanins, proanthocyanidins, flavonols, flavan-3-ols, and nonflavanoid compounds. CP had the highest malvidin-3-glucoside coumarate in grape must followed by MP and HP. RDI increased malvidin-3-glucoside-acetate by 32% compared to SDI. Catechin in grape seeds was 23% higher for SDI treatments than for RDI. This project provides applied production information for red winegrape growers in hot climates.

Funding Support: American Vineyard Foundation

Effects of Rootstock Selection and Irrigation Stress on Yield and Phenolic Composition of Zinfandel in Hot Climate

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The objective of this study was to evaluate the interactive effects of rootstocks (Freedom and Salt Creek) and phenology-based irrigated treatments on Zinfandel. Irrigation treatments were sustained deficit irrigation (SDI) where vines were maintained at a midday leaf water potential (Ψ) of -1.2 MPa and were irrigated to 80% of evapotranspiration (ET_0) from budbreak until harvest, and regulated deficit irrigation (RDI) that received 80% of ET_0 from budbreak to fruit set, whereafter 50% ET_0 was replaced to maintain (Ψ) at -1.4 MPa until veraison, but not thereafter. Treatments were arranged factorially with four replicated blocks. Canopy architecture, microclimate, yield components, yield efficiency, exhaustively extracted skin and seed, and partially extracted phenolics of grape must were measured. There was no effect of rootstock or irrigation on canopy architecture or microclimate. Berry size, cluster counts, and yield were not affected by irrigation or rootstock selection. Salt Creek and RDI treatments increased TA at harvest by 2% and 3%, respectively. Ravaz index and leaf area to fruit ratios were not affected by treatments. The phenolics measured included anthocyanins, proanthocyanidins, flavonols, flavan-3-ols, and nonflavanoids. Concentrations of gallic acid and petunidin-3-glucoside-acetate in grape marc were 62% and 68% higher in Salt Creek than in Freedom. Petunidin-3-glucoside-acetate in grape marc was 68% higher in the RDI treatment than in SDI. Concentrations of gallic acid in seed extracts were 49% higher in Salt Creek than in Freedom. Concentrations of catechin and myricetin in grape seed extract were 18% and 31% higher in Freedom than in Salt Creek. This project provides applied information to growers of red winegrapes in hot climates that yield and phenolic composition were maintained or increased depending on rootstock selection and phenology-based RDI application without adversely affecting yield.

Funding Support: American Vineyard Foundation

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**Effect of Undervine Vegetation Management on Vine Vigor,
Fruit Composition, and Wine Composition**

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This study is investigating what happens when undervine vegetation management using herbicide is replaced either by mowing or cultivation in Merlot, Syrah, Sauvignon blanc, and Pinot noir vineyards in New Zealand in order to increase the economic and environmental sustainability of the wine industry. Results from the first year of the trial at three of the sites show only small effects of treatments on water potential, nutrient status, and vine vigor, suggesting that allowing undervine vegetation does not compete heavily with vines for water or minerals. There were significant differences at the Merlot site in gap percentage and leaf area, but rarely in water potential, suggesting that subtle water status differences can lead to significant effects on canopy growth. At harvest, there was only a decrease in yield at the Merlot site, and this decrease was due entirely to smaller berries, as berry number per cluster and cluster number per vine were not different between treatments. Smaller berries are generally considered better for red winemaking, and this reduction in yield can make costly hand fruit thinning unnecessary. There were very few differences in basic wine composition parameters, even in the Merlot wines, suggesting no negative effect of undervine competition on fruit ripening. GC/MS analysis of the Sauvignon blanc wines showed a difference in IBMP content, with the herbicide treatment having significantly more than the mowing treatment, and the cultivation treatment intermediate between the two. The data show that impact aromas in wines can be manipulated by simply changing the undervine vegetation management, without sacrificing yield or negatively affecting other wine compositional parameters.

Funding Support: New Zealand Winegrowers

**Unraveling the Relationship between Grape Extractability and
Wine Composition**

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The concentration of phenolic compounds (particularly tannin) in grapes is an important factor in defining the texture of red wines and is influenced by a wide range of factors. A number of different methods are available for determining the phenolic profile in grapes and wine, but few studies have directly explored the relationship between grape phenolic profile and wine composi-

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tion. Wine tannin concentration is dependent not only on grape tannin concentration but also on the extractability of those tannins in the winemaking process. Studies have shown that sample preparation methods can significantly influence the extractability of color and tannin components from grapes. A detailed study carried out at the AWRI compared “total” anthocyanins and tannin measured from extraction (50% ethanol) of grape homogenate with actual levels extracted during fermentation of those grapes. The phenolic profiles of Cabernet Sauvignon and Shiraz grapes, from various regions within Australia, were measured before vinification in 1 kg lots. Wines were subjected to phenolic profiling using reference wet chemistry methods and using spectral calibrations (developed through the AWRI WineCloud). When considered on a varietal basis, total tannin levels measured in the grape homogenate samples correlated with those measured in the wines, but were noticeably higher than the actual concentrations achieved through vinification. The overestimation of tannin concentrations in grape homogenate extracts was greater in the Cabernet Sauvignon variety than in Shiraz. On the other hand, correlations between grape and wine anthocyanins and wine color density were strong, independent of grape variety. Rapid measurement techniques, such as the WineCloud, can already be used to identify the “total” potential of grapes; these latest findings suggest that a more wine-like extraction method may provide a stronger correlation with the ultimate phenolic profile of the wines.

Funding Support: Australian Wine Research Institute

Soil Geochemistry Fingerprints of Texas Hill Country Syrah Vineyards: Implications for Wine Differences?

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The characteristics of wine are attributed to the grape variety, the location where fruit is grown, and decisions made by the winemaker. The differences between wines from different locations may be due to aspect of the property, soil texture, or differences in soil geochemistry. This project focuses on the correlation between soil and wine characteristics of fruit from different soils. The elemental components of the soil can impact vine nutrition and vine vigor, thus influencing fruit size and intensity. The goal of this experiment was to produce fingerprints of different soils collected from six Syrah vineyards (with four replicate vines) selected from the Texas Hill Country. Soil geochemical fingerprints were created using inductively coupled plasma–optical emission spectroscopy (ICP-OES), in which soil samples were treated with plasma to yield ion particles of the different elements found in the soil. The results of the ICP-OES indicate that vineyards in the same region can be geochemically quite distinct. Our next step will be to analyze the geochemistry of each

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Viticulture – CONTINUED

single vine batch of Syrah wine from each location using ICP-OES to see if the geochemical fingerprint can be followed to the wine. We will also evaluate wine chemical characteristics and aroma against the soil geochemistry using multivariable statistics to determine if particular soil geochemical fingerprints correlate with particular wine characteristics.

Funding Support: USDA NIFA MSI grant

Temperature Profiles of Central Coast Vineyard Regions

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Grapevines are highly malleable plants which can be trained to grow over a wide range of practical trellising heights. The particular choice of trellis height may significantly influence the temperature microclimate experienced by the vines, particularly with regard to early or late season frost temperatures and extreme summer heat temperatures. To assess how air temperatures varied over a range of locations and weather conditions on the Central Coast, seven custom weather stations were installed in 2013 to measure air temperatures at eight heights between 0.3 m and 2.4 m above the ground surface, at intervals of 0.3 m. Some locations exhibited strong stratification (inversion) of the air temperatures on cold nights, indicating potential benefits for frost protection; temperature differences measured between 0.3 m and 2.4 m heights were as great as 5°C under the most heavily stratified conditions. Other locations commonly formed little to no temperature stratification at night due to air movement. Cumulative degree-days over the April to October growing season diminished with increasing height above the ground surface, with the 2.4 m height typically accumulating about 250 degree-days less than the 0.3 m height (base 10°C). The results of this first-year study indicate the potential useful information that may be obtained by conducting such detailed temperature analysis as a part of the vineyard site assessment process. Such information can be used to guide vine trellising decisions to potentially select for the optimum temperature conditions.

Funding Support: American Vineyard Foundation

*indicates corresponding author

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Viticulture – CONTINUED

Effects of Crop Loads and Vineyard Sites on the Winter Hardiness of Sauvignon blanc and Merlot

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In the Okanagan Valley in British Columbia winter temperatures occasionally dip below -20°C , causing serious injury to *Vitis vinifera* grapevines. Understanding the effects of management practices on grapevine winter hardiness could be important in minimizing the risk of winter injury. Experiments were established to assess the effects of crop load on bud hardiness of Sauvignon blanc and Merlot at several sites in the Okanagan Valley. Crop loads were adjusted by shoot and cluster thinning before bloom to an average of 1 or 1.5 clusters/shoot. At each site the higher crop load treatment increased the number of clusters per vine by 35 to 60%, and yield by 19 to 60%, and reduced juice soluble solids by 1.1 Brix in Merlot and 0.8 Brix in Sauvignon blanc. Bud hardiness was assessed at two-week intervals (Nov 2012 to Apr 2013) by testing bud numbers three to seven from harvested canes. Bud hardiness and the mean, minimum, and maximum temperatures varied by site. The higher crop load treatment increased bud hardiness in the Sauvignon blanc vines across all sites and sample dates by 0.6°C . A similar trend was found for Merlot with an average increase in hardiness of 0.4°C . These results indicate that nonexcessive increases in crop load may enhance bud hardiness. An analysis of the hardiness response to temperature across sites indicated that minimum daily temperatures had a greater impact on bud hardiness than did daily maximum temperatures.

Funding Support: Agriculture & Agri-Food Canada

Characterizing Cold Air Pooling in Vineyard Depressions and Scouring Using Frost Fans

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Understanding the growth of cold air pools in vineyard depressions during frost or freeze events can be useful in determining the necessity and timing of fan operation to protect vines from freeze damage. Using 3-dimensional temperature sensor meshes and volumetric analysis software, the shape, expansion, and temperature profile of cold air pools at three vineyard sites were characterized. The shape and filling pattern of the cooling air was consistent among overnight episodes at each site, but episodes varied in rate of filling and minimum temperatures reached. Disruptions in the filling pattern, likely caused by wind gusts, led to warmer minimum temperatures than expected from the

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Viticulture – CONTINUED

initial filling pattern. Without disruption, vertical temperature gradients of 0.6°C/m were observed in the cold air pool. Frost fan operation for one hour scoured out cold air from the depression and substantially reduced temperature variations within the air volume. These results reveal that some characteristics of cold air pools are consistent and useful in siting frost fans; however, ongoing temperature monitoring at key locations and heights during expected episodes can be used to optimally time frost fan operation to increase efficiency and minimize vine damage.

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

Anatomical Features of Grape Berries Frontenac and Frontenac Gris with Novel Anthocyanin Accumulation Patterns

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Synthesis of berry anthocyanins takes place in a mature organ with an organized arrangement of specialized tissues. In teinturier (dyer) varieties of grapes, it is known that anthocyanin synthesis is activated in cells across much of the berry tissue. The mature berry of Frontenac (*Vitis riparia* x Landot 4511) is entirely red and has thus been classified as a teinturier grape. Frontenac Gris, a single-cane sport of Frontenac, has an apparent colorless pulp. After examination of 2 mm-wide crosssections of both cultivars under epifluorescent microscope, we found that while both contain anthocyanin inclusions in the epidermal cells, they do not contain organs for anthocyanin synthesis in the cells beneath the epidermis. Nonetheless, red/pink pigment is seen within the cytoplasm of these cells. A transcriptome analysis of Frontenac berry skin and pulp showed a large number of anthocyanin biosynthesis genes were upregulated in the berry skin in relation to the pulp. The majority of genes with greater than two-fold upregulation in the skin of ripe berries were related to anthocyanin glycosylation. Observation of dissected berries during ripening shows that anthocyanin accumulation begins in the epidermis. This finding is in contrast to ripening patterns seen in other teinturier varieties, where anthocyanins first develop in the hypodermal cell layers. We suggest a passive transport mechanism exists which allows the transfer of pigment across the cell membrane in Frontenac and Frontenac Gris, accounting for their colored flesh. We theorize that the solubility of the glycosylated anthocyanins seen in hybrid grape cultivars may allow them to more easily pass through the cell membranes. In addition, we measured the thickness of the cell wall and the cuticle thickness in order to compare these features to other cultivars with known resistance to *Botrytis cinerea*.

Funding Support: USDA/NIFA Specialty Crops Research Initiative and Minnesota Agriculture Experiment Station

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Viticulture – CONTINUED

Impact of Nitrogen Fertilization Methods on Vine and Berry Nitrogen Status under Intensive Vineyard Cover Cropping

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Under-trellis cover crops have become more prevalent in East Coast grape-growing regions to minimize the potential for erosion and to help regulate grapevine size and vigor. Previous research has demonstrated that these companion cover crops can result in increased competition for soil nitrogen, leading to decreased vine nitrogen status and berry yeast-assimilable nitrogen. The objective of this work was to determine the effects of a range of nitrogen fertilization methods on vine and berry nitrogen parameters of cover-cropped grapevines. The effects of selected fertilization practices on Sauvignon blanc, Merlot, and Petit Manseng vines (*Vitis vinifera* L.) grown with companion cover crops were studied in northern Virginia, beginning in 2011. Data collection was taken in three years at the Sauvignon blanc site and two years at the Merlot and Petit Manseng sites. Sauvignon blanc petiole nitrogen, leaf chlorophyll content, and berry yeast-assimilable nitrogen at harvest were most affected by a high rate of soil-applied calcium nitrate and season-long foliar fertilization with urea; however, the foliar fertilization was most effective at increasing the concentration of individual amino acids measured in berries at harvest. Merlot berry yeast-assimilable nitrogen, petiole nitrogen, and leaf chlorophyll content were most affected by a high rate of soil-applied calcium nitrate and by establishing a clover mix as the under-trellis cover crop. Petit Manseng berry yeast assimilable nitrogen at harvest was increased in response to two postveraison foliar applications of urea, corresponding to an increased concentration of individual amino acids. We identified fertilization practices capable of overcoming the competition for nitrogen uptake between established cover crops and grapevines, such that adequate berry yeast-assimilable nitrogen levels were achieved. Our results warrant exploration of a combined soil and foliar-applied nitrogen fertilization approach to augment overall vine nitrogen status under intensive vineyard floor cover cropping.

Funding Support: Virginia Wine Board, USDA/NIFA Specialty Crops Research Initiative agreement #2010-51181-21599

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Napa and Sonoma Berry Report

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The purpose of this study was to identify relationships between different viticultural methods associated with berry size. 2010 and 2011 were very wet years in the Napa and Sonoma region and this report looks to understand the rate of change in growth of berries starting at veraison through harvest to see if there were noticeable differences in berry sizes. Vineyards in Napa and Sonoma have major differences in variance of row orientation from vineyard to vineyard. This study takes those different orientations into account as well as the differences in clones, rootstocks, elevations, and pruning methods to determine how they affect the size of a berry. We did comparisons within appellations of like clones, rootstocks, and viticultural methods, as well as comparisons of different appellations. We used statistical analysis on berry measurements to see if anything unique stood out that could provide information for future vineyard plantings. The findings led to interesting data regarding differences in these vineyard methods and how they affect wine berry size.

Funding Support: Robert Jordan

Influence of Soil Characteristics on Wine: Comparison of Syrah Wines from Six Texas Hill Country Vineyards

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Viticulturists are aware that topography and soil characteristics such as soil texture (percent sand, silt, and clay) can have a profound impact on wine quality. Shallow soils or soils high in sand may be lacking in water and nutrients, but soils that are too deep or with a high clay content may create too much vigor in the vine and also reduce wine quality. Our goal was to establish if soil texture impacts wine character and vine vigor differently across six Syrah vineyards in the Texas Hill Country. Soil and fruit samples were gathered from four replicate vines across six vineyards with distinct soils and single-vine wine batches were produced. By means of a principal components analysis (PCA), soil texture parameters of sand, silt, and clay were compared to viticulture parameters of stem weight (a vigor parameter) and basic juice and wine quality parameters such as pH, Brix, and yeast-available nitrogen (YAN). Our initial PCA showed that soil texture parameters could be linked to viticultural parameters such as stem weight. We are currently analyzing data to determine if soil texture and vine vigor parameters can be linked to specific aroma and taste characteristics for the Texas Hill Country vineyards we evaluated.

Funding Support: USDA NIFA MSI grant: Rocks to Wine

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Viticulture – CONTINUED

A Greater Understanding of Nutrient Supply for Pinot noir Production

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Previous research employing own-rooted Pinot noir grown at varying levels of nitrogen (N), phosphorus (P), and potassium (K) supply in a pot-in-pot vineyard indicated that N was the key driver of vine growth, yield, and berry metabolite profiles. Findings from the first two years (2012 and 2013) of a new trial using grafted Pinot noir vines with similar alterations of N, P, and K supply also show that N influences vine performance to a greater extent than P or K. Vines receiving the lowest rate of P began showing postharvest stress symptoms and vines receiving the lowest rate of K had exceptionally low petiole K concentrations and reduced must pH in 2013. These findings suggest that a robust test for P and K stress on vine productivity will be possible in the next growing season. The critical findings thus far in the new trial are that: only N supply reduced vine growth or yield and no nutrient altered flowering parameters; low N supply greatly reduced must YAN and fermentation rates sharply declined; low P supply reduced must P levels without altering the rate of fermentation; must YAN was well correlated with leaf blade N status over both years, but the relationship to petiole N differed by year; lower N supply altered the concentrations of two-thirds of the aroma volatiles detected in 2012 wines with greatest effect on yeast-derived esters and higher alcohols; and P and K supply had only minor effects on wine aromas. Our results indicate that N supply has a greater overall impact on Pinot noir productivity and presumably wine quality than P or K supply because it has a wider influence on vegetative growth, berry chemistry, and fermentation kinetics. New nutrient guidelines for Pinot noir will be derived from this comprehensive vine to wine study.

Funding Support: USDA-ARS, Oregon Wine Research Institute

trans-2-Hexenal Affects the Resveratrol Accumulation in Leaf Discs and Berry Skins

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trans-2-Hexenal (*t*-hexenal), one of the green leaf volatiles, is a physiologically important molecule that mediates the plant defense system. The effect of *t*-hexenal vapor treatment on the resveratrol accumulation in the leaf discs and berry skins was investigated in Muscat Bailey A (*Vitis labruscana*) and Ryuukyuganebu (*V. ficifolia* Bunge var. *ganebu*). Pretreatment with *t*-hexenal stimulated resveratrol accumulation in response to UV-C irradiation in the leaf

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Viticulture – CONTINUED

discs of Muscat Bailey A and Ryuukyuganebu. The effective concentration of *t*-hexenal depended on the grapes and the leaf developmental stages (the node position). In Muscat Bailey A, *t*-hexenal did not stimulate the resveratrol accumulation in the grape skins regardless of their developmental stages. In Ryuukyuganebu, however, *t*-hexenal stimulated the resveratrol accumulation in response to UV-C irradiation in the berries at veraison. Interestingly, in the ripening berries of Ryuukyuganebu, *t*-hexenal indirectly and directly stimulated resveratrol accumulation in the skins. The levels of resveratrol elicited by *t*-hexenal augmented with increasing in *t*-hexenal concentration. The resveratrol levels in the skins were higher in the treatment with 86.2 μM *t*-hexenal than that in UV-C (285 $\mu\text{W}/\text{cm}^2$) irradiation for 15 min. These results indicate that *t*-hexenal has priming effect on the resveratrol accumulation in response to UV-C irradiation in leaf discs of Muscat Bailey A and Ryuukyuganebu. In Ryuukyuganebu ripening berries, *t*-hexenal shows not only a priming but also an elicitor effect on the resveratrol accumulation in the skins.

Funding Support: Osaka Prefecture University

The Utility of Growing Degree Days as an Index in Viticulture

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Growing degree days is one of the most frequently used weather-based indices for assessing the suitability of a grape variety to a particular location. Growing degree days is the day degree accumulation for a particular location given a threshold temperature over a specified time frame (growing season). Growing degree days serves to simplify complex temporal temperature patterns at a single location. As such, its utility (like any index) is driven by two often antagonistic concepts: simplicity and accuracy of calculation and its predictive power. The goal of this research is to assess the utility of growing degree days as an index of suitability for winegrape selection. In our first objective, we determine the error in growing degree day estimates likely to occur using weather data with different spatial and temporal resolutions. Estimates of growing degree days vary by as much as 5% due to annual variation and 10% depending on the temporal resolution of the data. Our results also indicate that 90% of the variation in an estimate of growing degree days can be explained by elevation, latitude, and longitude. In a second objective, we assess the predictive ability of growing degree days for viticulture. In viticulture the concept is most often used to understand which varieties can reach maturity and which cannot. We conclude that data availability drives the use of growing degree days as a concept in agriculture and that the interpretation of growing degree days for variety selection requires an understanding of the method of calculation and the resolution of the data used.

Funding Support: Texas AgriLife Extension Service

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Viticulture – CONTINUED

Effect of Nitrogen Application Rate and Timing on Methoxyypyrazine Development in Cabernet Sauvignon Grapes

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Methoxyypyrazines are nitrogen-containing compounds that produce vegetative aromas in winegrapes. A three-year study investigated the effects of nitrogen application rate and timing on methoxyypyrazine metabolism during fruit development to determine if it is influenced by vine nitrogen status. Of five methoxyypyrazines measured, isobutylmethoxyypyrazine was the most abundant and was the only one found at sensory threshold concentrations. Varying nitrogen application rate and timing resulted in a 40% difference in isobutylmethoxyypyrazine among treatments. High rates of nitrogen applied at budbreak and berry set resulted in high levels of berry isobutylmethoxyypyrazine throughout development. Low nitrogen rates and later nitrogen applications at berry set and veraison resulted in low isobutylmethoxyypyrazine. High nitrogen application at veraison did not affect isobutylmethoxyypyrazine content. Fruit with higher isobutylmethoxyypyrazine early in the season remained higher through to maturity. Cluster exposure, canopy density, and berry size and maturity were not significantly different among treatments. Climatic conditions affected methoxyypyrazine biosynthesis between years but did not affect the relative differences among treatments. In this study methoxyypyrazine content was more influenced by vine nitrogen status than canopy structure, vine vigor, or fruit maturity.

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

Profiles and Antioxidant and Antimicrobial Activities of Fifty-Eight Muscadine Varietal Skin and Seed Polyphenols

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The muscadine grape (*Vitis rotundifolia* Michx.) industry in the southeastern United States has expanded in recent years as more varieties become available. To provide nutraceutical information for this industry, 58 muscadine varieties harvested from the research vineyard at Florida A&M University were evaluated for their phenolic profiles and antioxidant and antimicrobial activities during two growing seasons (2012, 2013). Three commercial grape varieties were used for comparison. Results showed that the total phenolic content and antioxidant activities of all muscadine varieties, based on dry weight, were higher in seeds than skins, and 80% of them were higher than commercial grapes. For seeds, total phenolic content on average ranged from 22.5 (cv. Sugar Pop) to 72.0

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Viticulture – CONTINUED

(cv. Florida Fry) mg GA/g DM, and antioxidant activity ranged from 178.2 (cv. Sugar Pop) to 619.7 (cv. Florida Fry) $\mu\text{mol Trolox/g DM}$. For skins, total phenolic content on average ranged from 10.1 (cv. Scartlett) to 30.0 (cv. Janebell) mg GA/g DM, and antioxidant activity ranged from 83.6 (cv. Scartlett) to 221.2 (cv. Janebell) $\mu\text{mol Trolox/g DM}$. The main compounds identified in seeds included gallic acid, catechin, epicatechin, epicatechin gallate, pentagalloylglucose, and ellagic acid. Skins contained gallic acid, anthocyanins, epicatechin, ellagic acid, resveratrol, and quercetin. Significant season and varietal differences were found in seed (skin) total phenolic content but not in their phenolic profiles. Muscadine polyphenols also exhibited a broad spectrum of antimicrobial activity against *Staphylococcus aureus*, with MIC_{50} 36.0 to 72.2 $\mu\text{g/mL}$ for seeds and 54.3 to 102.8 $\mu\text{g/mL}$ for skins. Overall, total phenolic content showed significant correlation to their total antioxidant ($r = 0.94$) and total antimicrobial ($r = 0.82$) activities. Access to this information would be useful to muscadine breeders, growers, and processors, allowing them to make decisions on which cultivars offer potential added value to a waste stream by producing functional ingredients.

Funding Support: College of Agricultural and Life Sciences—University of Florida

Comparison of California Heritage Zinfandel and Primitivo Grapevine Selections in Napa Valley, California

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Seven clones of Zinfandel (*Vitis vinifera*) and one clone of Primitivo were compared for observable, morphological differences. The clonal selections were gathered from heritage vineyards of at least 60 years of age throughout the state of California. The selections were all grafted onto St. George rootstock and planted at the University of California, Davis, Oakville Experimental Station. The objective of this study was to determine whether significant differences in fruit composition existed among these clonal selections. We measured cluster weights, berry weights, berries per cluster, and seeds per berry to assess morphological differences of the grape cluster architecture. Juice composition was examined by measuring soluble solids, pH, titratable acidity, YAN, and malic acid. Phenolic composition of the grape skins was examined using high-performance liquid chromatography. Finally, pruning weights were measured in the following winter season. Preliminary results demonstrate that most parameters show no statistical differences between the clones. However, we observed fewer berries per cluster in the Primitivo clone, and this trait may be linked to the higher degree of soluble solids that was also observed in this clone. The complete set of morphological and fruit quality data will be presented, as well as clonal diversity implications.

Funding Support: Zinfandel Advocates and Producers

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Viticulture – CONTINUED

Evaluation of Mechanically Applied Heat in Syrah and Merlot

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In cool climates, heat accumulation is critical for fruit ripening. However, can added heat exposure improve fruit quality in warm summer climates like those of eastern Washington? In 2013, a directed-heat application machine (Agro-thermal Systems) was evaluated for its ability to improve vine growth and fruit quality in commercially grown *Vitis vinifera* Syrah and Merlot in Paterson, WA. Heat was applied via burning of propane; angled vents direct the heated air to the vine fruit zone and canopy. The apparatus was driven down vineyard rows at 4 mph. Rows were treated on a weekly to biweekly basis with heat treatments during: (i) bloom only; (ii) veraison only; (iii) both bloom and veraison; (iv) from budbreak to harvest; and (v) a no-heat applied control. Data collected included the timing of phenology, percent fruit set, duration and level of heat exposure of the fruit and canopy, and final harvest juice sugar, titratable acidity, and pH. Air temperature at the heating vents was approximately 115 to 120°C. However, by the time the air reached the canopy, air temperature was approximately 50 to 55°C. As a result, the typical increase in leaf or cluster temperature was about 5 to 10°C above ambient for only 10 to 20 seconds. Heat application did not increase number of berries per cluster or the fruit set compared to no-heat treatment. Heat application did not enhance or abridge key vine phenology stages, did not increase sugar accumulation, and did not alter juice TA or pH. Results indicate that heat application of this form does not improve vine phenology and in-field measured aspects of fruit quality in climates such as irrigated eastern Washington.

Funding Support: Washington Wine Advisory Board, Altria Group, Inc., Ste. Michelle Wine Estates, WSU Viticulture and Enology Program

Determining the Effect of Regulated Deficit Irrigation on Cabernet Sauvignon in Washington

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A trial was conducted to understand the role of regulated deficit irrigation (RDI) in vine growth and yield of Cabernet Sauvignon in eastern Washington. Four different levels of irrigation were applied: 25% ET_c (treatment I), 25% ET_c preveraison and 100% ET_c postveraison (treatment II), 70% ET_c (treatment III), and 100% ET_c (treatment IV). Stem water potential, gas exchange rates, shoot growth, and yield components were measured from

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bloom to harvest in 2011, 2012, and 2013. The growing season in 2011 was cooler (1486 GDD), 2012 was average (1640 GDD), and 2013 was warmer (1789 GDD) than average. Stem water potential measurements indicated that the vines under treatment I were water stressed throughout the growing season, those under treatment II were stressed only during preveraison, while those under treatment III and IV were nonstressed during the entire season. In general as irrigation level (ET_c) increased so did the gas exchange rates during pre- and postveraison in all three years. Shoot growth and yield components for treatments II, III, and IV were similar and significantly higher than those in treatment I in all three years. Early season shoot measurements indicated a carryover effect due to RDI treatments from previous years. Treatment I expedited color accumulation; however, the number of clusters per vine, yield, and average cluster weight were significantly lower compared to treatments II, III, and IV. Changing the irrigation regime from 25% ET_c preveraison to 100% ET_c postveraison (treatment II) reduced water stress, increased gas exchange, and improved yield compared to just 25% ET_c (treatment I). Treatment II had the potential to improve yield while at the same time saving irrigation water.

Funding Support: Washington State University, Washington State Grape and Wine Research Program, Altria Group, Ste. Michelle Wine Estates

Functional Analysis of Pierce's Disease Resistance Genes from *Vitis arizonica*

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Pierce's disease (PD) is a deadly disease of grapevines caused by the bacterial pathogen *Xylella fastidiosa* (Xf). Resistance to PD is present in North American *Vitis* species. Resistance from *V. arizonica* accession b43-17 has been mapped as a single major locus (*PdRI*) on to chromosome 14. The physical mapping of the *PdRIb* locus allowed the identification of potential candidate resistance gene(s) at this locus. We cloned candidate genes *PdRIb.1*, *2*, *4*, *5*, and *6* and generated five gene constructs that were used to transform embryogenic callus of *V. vinifera* Chardonnay and Thompson Seedless and *V. rupestris* St. George via *Agrobacterium tumefaciens*. Transgenic plants of Chardonnay carrying the candidate genes under the control of the 35S CaMV promoter were acclimated for testing against Xf in the greenhouse. Gene expression levels were analyzed by qPCR. Independent lines of each gene were needle-inoculated in two basal nodes with 10 μ L of 10^8 cfu/mL of Xf Beringer strain. Symptoms based on leaf scorch and cane maturation were scored three months after inoculation, with some transgenic lines showing scores comparable to those of resistant biocon-

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trols. PD-resistance analysis based on Xf counts through ELISA is underway. Some lines transformed with *PdR1b.6* displayed reduced size and altered leaf morphology, indicating that the constitutive expression of this gene is affecting normal growth. We will present results from the screening of 10 independent lines of each gene in Chardonnay and will discuss their significance in relation to their transgene expression levels.

Funding Support: CDEA Pierce's Disease Board

Molecular Analysis of Phylloxera Present in Argentinean Vineyards

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In Argentina, grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is not considered a main threat to the grape industry, even though most of the vines are cultivated on their own roots. However, recent changes in viticultural practices such as drip irrigation could alter this situation. The aim of this study was to determine the population structure and diversity of phylloxera in Argentina and compare it to fingerprint profiles from the United States and other countries to determine from where Argentine phylloxera were introduced. A total of 112 samples were collected from roots of *Vitis vinifera* cultivars grown in different localities of the provinces Mendoza, San Juan, La Rioja, and Río Negro. DNA was extracted using the Chelex method and amplified at 21 microsatellite markers. The data was analyzed using DARwin v.5 and Structure 2.3.4 software. Samples from Argentina divided into two distinct groups. The largest group consisted of 87 samples from Mendoza, San Juan, and La Rioja. Three distinct genotypes were identified and they grouped closer to the California biotype A reference and samples from Peru. The second group consisted of samples from northwest Mendoza, Río Negro, and one sample each from San Juan and La Rioja. There were seven unique genotypes and they grouped closer to the California biotype B reference. The Argentinean samples did not group close to samples from the eastern United States, Europe, or other South American countries. These results indicate that Argentine phylloxera are genetically diverse and that more analysis is required to understand the origin of strains and the impact this diversity will have on control strategies.

Funding Support: CONICET and Catena Institute of Wine

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Anthracoze Incidence in Different *Vitis labrusca* Cultivar and Rootstock Combinations in Humid Subtropical Climate

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Grapevine anthracnose is a severe disease that affects several grape production areas. It is considered a tropical disease, and therefore the evaluation of grape cultivars before vineyard planting is important for choosing the most suitable ones. Rustic grapes are an important option for vintners in South Brazil; however, high relative humidity and other conditions favor the occurrence of fungal disease in vines. The objective of this study was to evaluate the anthracnose incidence progress in grape leaves of three scion cultivars (Concord, Bordô, and BRS Carmen) in combination with three rootstocks (1103 Paulsen, VR 043-43, and IAC 766) in a place with favorable conditions for disease development. The experiments were carried out on ridges with conduction systems in semi-trellis. The disease incidence was assessed between September and March 2010/2011, 2011/2012, and 2012/2013 each 14 days, in the last 10 leaves in the apex of the plant. Six plants of each different combination cultivar rootstock were evaluated, totaling 54 plants. The area under the disease progress curve (AUDPC) in the first harvest showed cv. BRS Carmen as the most susceptible and cv. Concord the least. In the second and third season, cv. Bordô had the highest disease incidence (76.6%), differing statistically (p value <0.05) from the other two cultivars in the second year, but only from Carmen in the third. The unfolding of the interaction showed differences among rootstocks for BRS Carmen; IAC 766 was the most susceptible in 2010/2011 and VR 043-43 was the least in 2011/2012. However, in 2013, for the Concord cultivar, VR 043-43 was the most tolerant rootstock. All evaluated combinations showed disease symptoms with a high disease progress period coinciding with the greatest amount of precipitation. There was a disease increase between the years; it showed the importance of the initial inoculum amount and inefficient phytosanitary control. There was a rootstock effect for BRS Carmen, but this becomes masked if the disease incidence is very high, and Concord is the least susceptible cultivar.

Funding Support: CNPq CAPES

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Assessment of Pierce's Disease Resistant 87.5% *Vitis vinifera* Selections in Alabama

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Pierce's disease (PD) has prevented the production of high-quality *Vitis vinifera* grapevines in the southern United States. Recently, the University of California, Davis grape breeding program utilized conventional breeding methods to introgress PD resistance from native American species into elite *V. vinifera* wine and table grapes. Identification of a single dominant PD resistance gene (*PdR1*) allowed marker-assisted selection of resistant seedlings, followed by multiple cycles of backcrossing to *V. vinifera*. In 2010, three PD resistant selections, 502-10, 502-01, and 501-12, were established at the Chilton Research and Extension Center near Clanton, Alabama. The experimental vineyard utilizes a RCBD with six blocks and 5 vines per block and is trained to a vertical shoot-positioned trellis. Fruit was dropped in 2011 and the first commercial crop was produced in 2012. Our 2012–2013 results suggest early-season selection 502-10 had the lowest pruning weight in both years, while the late-season 501-12 had the highest. The three selections differed in total yield per vine. The late-maturing 501-12 produced the largest cumulative crop of 13.9 kg/vine, whereas the midseason 502-01 yielded 9.2 kg/vine. In both years, selection 501-12 produced the highest number of clusters per vine. Midseason selection 502-01 had the largest berries, while the late-season 501-12 produced the sweetest berries. Our second year results for the growth and development of newly developed PD-resistant 87.5% *V. vinifera* selections with different season of ripening are very promising. Research will continue in multiple seasons to fully evaluate their performance in the high PD-risk Alabama environment.

Funding Support: USDA-ADAI Specialty Crops Block Grant, Alabama Agricultural Experiment Station HATCH Grant

Role of Grape Mealybug in Grapevine Leafroll Disease Epidemiology in Northern California and Implications for Management

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Grapevine leafroll disease (GLD) occurs in every major grapegrowing region of the world. The disease is not typically lethal but does result in significant economic loss from a combination of factors, including a significantly shortened vineyard life. Symptomatic plants experience overall decreases in net photosynthetic rates postveraison, resulting in decreased fruit quality,

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pigmentation, and delayed maturity, as well as yield reductions of up to 40%. Of the serologically distinct viruses known collectively as grapevine leafroll-associated viruses (GLRaV), GLRaV-3 is the predominant species of concern. In northern California vineyards, the most commonly occurring vector of GLRaV-3 is the grape mealybug, *Pseudococcus maritimus*. This study documented the incidence of grape mealybug (GMB) and GLD in 10 vineyards in Napa County over four growing seasons (2010-2013). We recorded the number of GLD-symptomatic vines and the incidence of GMB in grape clusters in fall (September to October) of each growing season. A generalized linear mixed model describes the relationship between GLD and GMB, with the number of vines found with GMB in the previous season a highly significant variable in the model. A regression tree produced similar results: disease pressure (number of vines with GLD carried over from the previous season) and GMB populations in the previous season describe the differences in rates of disease spread between experimental blocks. The significance of disease pressure and vector populations has important implications for management of grapevine leafroll disease in California vineyards.

Funding Support: American Vineyard Foundation, Viticulture Consortium West

Potential to Eradicate *Xiphinema index* Using the Bioantagonistic Rootstock O39-16

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Initial reproduction studies of *Xiphinema index* on O39-16 showed that populations decreased over time. In addition, the alternative host range of *X. index* is limited and does not seem to include many common vineyard weeds. This study was conducted to determine the most effective sampling method to recover *X. index* and to evaluate the possibility that the nematode can be eradicated over time from vineyards that have been planted with O39-16. Two sampling methods were used (shovel and Oakfield tube) and the nematodes were extracted and identified. Pearson's test was performed to determine that there was a poor correlation between the two methods. Subsequent sampling used the shovel method. The populations of *X. index* and *X. americanum* on O39-16 were compared with adjacent populations on 3309C and 110R, both susceptible to *X. index* feeding. Samples were collected from beneath drip emitters on three dates, and on each date the same drip zones were sampled. Nematodes were extracted and identified. Very few *X. index* were recovered from O39-16; most samples were devoid of *X. index*. Significantly fewer *X. index* were recovered from O39-16 than from either 3309C or 110R. There was a tendency for O39-16 to have more *X. americanum* than either 3309C or 110R, although the differences were usually not significant. To verify the absence of *X. index* on O39-16, soil pits were dug alongside previously sampled

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vines. Samples were collected at 25 cm, 50 cm, and 100 cm and nematodes were extracted and identified. Although the differences were not significant, there was a trend for fewer nematodes at increasing depths. In conclusion, the likelihood that *X. index* can be eradicated through the use of O39-16 is high. However, these results need to be verified in other vineyards, especially those planted solely on 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, Louis P. Martini Endowed Chair for Viticulture

Ethanol and Acetic Acid Content of Sour-Rotted Winegrapes and Disease Control with Antimicrobial and Insecticide Sprays

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Sour rot is a poorly defined disease, characterized by the smell of acetic acid emanating from grapes decaying on the vine and typically associated with large populations of fruit flies (*Drosophila* spp.) around affected clusters. Sixteen samples of grape bunches with sour rot were collected from 12 vineyards in the Finger Lakes region of New York in October 2013 and analyzed for acetic acid and ethanol content. The mean acetic acid content in expressed juice was 2.41 gL⁻¹, twice the recommended volatile acidity maximum, and the ethanol content averaged 0.42% v/v. Concentrations of ethanol and acetic acid were inversely proportional among individual samples, suggesting that alcoholic fermentation of decaying berries followed by conversion of ethanol to acetic acid was occurring on the vine. The rinsate from a composite sample of these bunches revealed large populations of *Saccharomyces* spp., identified by sequencing of the ITS region. A replicated split-plot control trial was established in a vineyard of the disease-prone interspecific hybrid cv. Vignoles, examining four to five weekly sprays of the antimicrobials potassium metabisulfite or copper hydroxide applied to rows concurrently treated or untreated with the insecticide acetamiprid (Assail), beginning at approximately 15 Brix. Four different programs of antimicrobials applied to vines without insecticide had little effect (mean of 9% control relative to the untreated check), whereas they provided a mean of 50% control on vines also receiving insecticide; insecticide without microbials provided insignificant control. We hypothesize that sour rot results from the initial fermentation of juice from injured berries to ethanol by *Saccharomyces* and/or other yeasts, followed by its oxidation to acetic acid by known bacteria; that *Drosophila* spp. are important vectors of these microorganisms; and that control of such components can reduce the disease. Current work is focused on better identifying the specific roles of the various factors involved.

Funding Support: NY Wine and Grape Foundation

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Promising Strategies for Managing Cotton Root Rot of Winegrapes in Texas

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As with any young and growing agricultural enterprise, the list of challenges to winegrape production in Texas increases as crop acreage expands. The destructive presence of the cotton root rot pathogen, *Phymatotrichopsis omnivora*, is a perfect example. Cotton root rot is a notorious plant disease affecting a wide variety of Texas crops for which disease control has been challenging. Two of the approaches being considered for cotton root rot control in grapes are fungicides and rootstock selection. Fungicide trials were initiated in 2011 at a commercial vineyard and an experimental vineyard, both with high disease pressure. Emphasis was placed on soil drench applications of flutriafol, a new chemical being used to control cotton root rot on cotton. Three other compounds are also being tested at one of the sites. After two years of evaluation in the experimental vineyard, data are still not sufficient to evaluate the efficacy of the treatments. However, in the commercial vineyard the mortality levels in the untreated control plots are increasing while some treated plots appear to be unaffected by the disease. Greater differences among the treatments are expected to be more apparent in the coming growing season (2014). In addition to the fungicide trials, the experimental vineyard has a planting of rootstock evaluations. Merlot and Cabernet Sauvignon have been planted on their own roots and on Dog Ridge (*Vitis champinii*) rootstock to test the putative tolerance to *P. omnivora*. These plots will continue to be monitored along with the fungicide tests. In addition, greenhouse experiments are underway with mini-rhizotrons in which grapevines are planted and inoculated with the pathogen in order to directly observe disease response on the roots. This tool is being tested in an attempt to expedite screening of the rootstocks and fungicide treatments under controlled conditions.

Funding Support: Texas Department of Agriculture Specialty Crop Block Grant Program

Functional Microclimate Size in Powdery Mildew Epidemiology and Impacts for Forecasting and Disease Control

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Powdery mildew (PM) causes major damage to winegrapes in California. Typical treatment consists of sulfur applications throughout berry development. The Gubler–Thomas model is used to estimate PM risk based primarily on temperature in order to identify intervals in which to treat the disease. The

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model cannot predict risk without accurate weather information yet weather station costs are often prohibitive to many growers. Virtual weather networks can interpolate data from existing weather stations and display it freely available online, offering an alternative to buying weather stations to determine PM risk and make recommendations. This study examines (1) the success of virtual weather networks in calculating risk as compared to corresponding observing weather stations in 10 vineyards in Lodi, California, and (2) the subsequent recommendations these risk-estimation methods have on PM control. This was accomplished by using a weather algorithm developed at Oregon State University that weights the output of nearby observing weather stations for proximity and elevation. Observing Adcon stations were assembled and deployed in each of the 10 vineyards. Virtual models did not include output from the newly installed observing stations in their calculations. In most sites in central and western Lodi, indices diverged up to 80% of the time, while in eastern Lodi, indices converged over 80% of the time. In all but one site, the virtual model underestimated the observing model. This divergence indicates that there is a difference between site-specific weather conditions and the averaged regional conditions in western and central but not eastern Lodi, indicating that microclimates affecting powdery mildew growth have a gradient from smaller and heterogeneous in western Lodi to larger and homogeneous in eastern. Future work will characterize microclimactic features and sizes that affect powdery mildew sporulation, including characteristics such as daily fluctuation in relative humidity and extreme heat events to improve algorithm function.

Funding Support: Subcontract of USDA-AFRI grant to Oregon State University

Efficacy of Biopesticide-based Programs on *Erysiphe necator* in *Vitis vinifera* Chardonnay

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The management of grape powdery mildew (caused by *Erysiphe necator*) can be difficult using conventional management programs due to constraints in managing the emergence of pathogen strains resistant to registered fungicides, but is far more challenging for organic programs due to the limitation of fungicide choices for disease management. To optimize the effectiveness of the spectrum of organic options for disease management, a combination of chemical and cultural disease management strategies is usually necessary. In recent studies, biopesticide-based fungicide programs combined with different timings of fruit-zone leaf removal (pre-bunch closure) were evaluated in 2013. The trial was conducted using a 3-year-old *Vitis vinifera* Chardonnay vineyard located at WSU-IAREC in Prosser, Washington. Fungicide programs using only plant and bacteria-based active ingredients and programs that combined those biopesticides with standard synthetic fungicides were evaluated. Leaf

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removal treatments were nested within the various fungicide programs. Irrespective of timing, leaf removal resulted in lower powdery mildew incidence and severity compared to treatments with no leaf removal. Early fruit-zone leaf removal significantly improved spray penetration ($F = 0.002$ to 0.02). Final harvest soluble solids and titratable acidity were significantly altered by the choice of biopesticide-based programs ($F = 0.0001$ and 0.009 , respectively). Even though results fell within industry-acceptable ranges, there was a direct correlation between higher disease levels and lower soluble solids/higher titratable acidity. Our results indicate that all biopesticide-based spray programs provide the same level of powdery mildew control. Superior programs combine synthetic products during bloom with biopesticides during lower-risk periods. In some cases, spray programs consisting of only biopesticides had a significantly higher incidence and severity of disease than the unsprayed controls ($F = 0.0001$). In high-pressure scenarios, a poorly designed spray program using fungicides of marginal efficacy results in similar or higher disease incidence than forgoing a season-long fungicide program.

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

Fungal Diseases in Grapevine: Phenotypic Assays on a *Vitis aestivalis*-derived Norton-based Population

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Fungal diseases are a primary obstacle in grape and wine production. Major diseases of grapevine include powdery mildew, downy mildew, and *Botrytis* bunch rot. European grapes (*Vitis vinifera*), from which most of the wine is made worldwide, are susceptible to these diseases whereas the majority of North American and few Asian varieties possess various levels of resistance. To study disease resistance, 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 progeny. This population was further expanded to 184 individuals by repeating the same crosses in 2011 and 2012. Using this mapping population, improved methods of phenotyping were developed under laboratory and greenhouse conditions to evaluate disease development for powdery mildew, downy mildew, and *Botrytis* bunch rot. Preliminary data on the segregation pattern in the mapping population for powdery mildew and *Botrytis* bunch rot has been obtained. Results from this study will be utilized to expedite the grape breeding programs in Missouri.

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Impact of Grapevine Red Blotch-Associated Virus on Performance of Three Winegrape Cultivars in North Coast Vineyards

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Grapevine red blotch-associated virus (GRBaV) was first identified in 2011 and subsequently shown to be the causal agent of red blotch disease. Diseased vines have been identified in several counties in California and in other states. In 2013, we evaluated the effect of GRBaV infection on canopy symptom development, fruit maturity, and yield components in three winegrape cultivars in vineyards located in Napa and Sonoma Counties. Chardonnay, Merlot, and Cabernet Sauvignon vines were monitored that were determined to be GRBaV positive or negative by qPCR assay as well as negative for all grapevine leafroll-associated viruses, vitiviruses, and nepoviruses. The number of symptomatic leaves was significantly greater in older than in younger leaf blades and older blades had larger estimated surface area (greater symptom severity) with red or chlorotic coloration in red and white varieties, respectively, than younger terminal leaves. Over 60% of the leaves in Chardonnay were symptomatic on 11 Sept with an estimated 34% of leaf area chlorotic in the basal region of shoots. Merlot had 29% red leaf area in the basal region on 1 Oct, while in Cabernet Sauvignon less than 3% of the leaf area in the basal region was red in mid-September. For all three cultivars, fruit maturity was delayed in GRBaV-positive vines. Total soluble solids (Brix) were significantly lower on all sample dates and at harvest juice samples in all cultivars had significantly higher malic acid in GRBaV positive vines as compared to negative vines. Mean berry mass was significantly greater on all samples dates in Merlot and on the final sample date in Chardonnay in GRBaV-positive vines. Reducing crop load in GRBaV-positive vines by approximately 35% at veraison in Chardonnay and Cabernet Sauvignon did not significantly improve juice chemistry at harvest when compared to infected vines with full crop loads.

Funding Support: American Vineyard Foundation

Grape Rootstock-Scion Interactions and Their Influence on Ripening Periods and the Initiation of Senescence

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Currently, rootstock decisions are primarily based on pest resistance factors and adaptation to soils or environmental conditions. Research continues to look for better resistance to a range of pests and diseases, but little work has been done to clarify a rootstock's ability to influence ripening periods and the

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initiation of dormancy of a scion. The main objective of this research was to determine the role grapevine rootstock parentage has on scion phenology: specifically, extended or shortened ripening periods, as determined by the initiation of senescence. This study involves commercial vineyard field trials at three locations and the development of a shadehouse assay for expedited senescence scoring. Rootstock choice was found to significantly influence scion internode length, shoot caliber above the second cluster, leaf persistence over the course of the season, and canopy density via light bar measures. These traits were used to evaluate and characterize rootstock influence on scion growth, development, and senescence. Determining the role parentage plays in rootstock-scion interactions and utilizing that knowledge in a rootstock breeding program will provide growers with rootstocks capable of providing longer seasonal growth allowing sugar and quality-based compounds to accumulate in berries or shorter seasons to avoid temperature or precipitation pressures.

Funding Support: California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, American Vineyard Foundation, CDEFA Improvement Advisory Board, California Table Grape Commission, and Louis P. Martini Endowed Chair for Viticulture and (scholarship funds), American Vineyard Foundation, CJ Alley Fellowship, Knights of the Vine, Wine Spectator, Kunde Scholarship, Orange County Wine Society, Margaret Mondavi Award, J. Pickett Agricultural Scholarship and Winkler Fellowship

Using a Rhizotron to Evaluate Deep and Shallow Rooting in Grape Rootstocks

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There have been several attempts to create containerized, nondestructive assay methods to look at root architecture in plants. However, few such methods have been developed to examine grape roots. The purpose of this study was to evaluate the utility of a greenhouse-grown rhizotron/rootbox assay for root architecture in grapes. A 25 x 50 x 2 cm plexiglass and wood rhizotron was used to observe the root architectures of the grape rootstocks Ramsey (*Vitis champinini*) and Riparia Gloire (*V. riparia*). Riparia Gloire is considered to have shallow rooting angles and Ramsey to have deep root angles. Previous studies have examined root angles from field nurseries and within backhoe pits. Rhizotrons offer an opportunity to examine grape roots in a two-dimensional space so that the angles are clearly observed. The two rootstocks were grown in the rhizotrons for four weeks on seven different cycles. Root systems were traced weekly with acetate sheets over their plexiglass front cover during the period of growth. Root and shoot dry weight were collected at the end of the fourth week. In addition, root girths were measured across a chosen transect. The cumulative root fraction graphs found that Ramsey was consistently more

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deeply rooted (6 cycles with $p < 0.05$) and Riparia roots were more shallow. Riparia had a greater density of root counts in the upper half of the rhizotron than Ramsey. There were no statistical differences or patterns seen in root dry weight or root dry weight fraction (root dry weight over total dry weight). Riparia had consistently higher shoot dry weights (4 cycles with $p < 0.05$). The results support existing notions of the deep and shallow rooting behaviors and demonstrate an effective and rapid way to measure rooting depths.

Funding Support: California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, American Vineyard Foundation, Cdfa Improvement Advisory Board, and California Table Grape Commission

Rootstock Trials for Central and Southeast Texas: Regions of Moderate to High Pierce's Disease Probability

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A rootstock trial was conducted in central Texas near the town of Tow from 2005 through 2007 to assess Pierce's disease (PD) field tolerance of ungrafted vines. Thirteen rootstock varieties were chosen, many of which are common stocks used in Texas or have potential for the region. Visual PD symptom ratings, pruned weight, and *Xylella fastidiosa* serological detection data are presented here. Results show a wide spectrum of response among rootstock varieties, as some rootstocks displayed minor PD symptoms and low detectable levels of *X. fastidiosa* while others had severe symptoms, high levels of *X. fastidiosa*, and vine death. Based on these results a grafted rootstock trial was initiated in 2011 with the objective of measuring the impact of rootstock selection on disease tolerance and vine performance. Five of the top performing rootstocks from the ungrafted study were included along with six additional rootstocks that have potential for the region. Three replicate sites were chosen to assess performance over a range of growing conditions: plots on fractured limestone in southwestern Texas (Real County), alluvial sandy-clay soil with high pH in central Texas (Gillespie County), and clay-loam soil in southeastern Texas (Austin County). Two plots with the scion variety Sangiovese (high fruit quality in central Texas) were planted in 2011 in Real and Gillespie Counties. The third plot with the scion variety Blanc du Bois (industry standard along the Texas Gulf Coast) was planted in Austin County in 2012. These companion plantings constitute the most significant evaluation of grape rootstock on the performance of winegrapes in the south in the past 25 years. In addition to their impact on scion survival of Pierce's disease, these trials provide insight on vigor, nutrient uptake, and tolerance to soil-borne pathogens as well as their influence on date of budbreak and crop maturity.

Funding Support: Texas Department of Agriculture Block Grant

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Sour Shrivel: A Paradoxical Ripening Disorder of Grape Berries

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Sour shrivel, a physiological ripening disorder, renders grape berries unsuitable for making wine. To determine its mechanistic basis, fruit organoleptic attributes coupled with morphology and structure and tissue organization in various organs of healthy and afflicted grapevines were examined using a range of microscopy techniques. Regardless of grape types, the sour shrivel symptoms were identical in all cultivars. As opposed to healthy berries, sour shrivel berries were flaccid, which compromised fruit quality attributes such as hexoses, nutrients, and various phenolic compounds including anthocyanins. Despite such extreme quality losses, the spatial architecture and integrity of various tissues in organs such as canes and the inflorescence framework of afflicted vines were remarkably similar to healthy grapevines with notable exceptions. Although the spatio-temporal pattern of various tissues determining the structure-function relationship in afflicted vines remained intact throughout the growing season, the functionality, especially of flows in vascular tissues (sieve tubes) started to slow and eventually ceased during ripening, as evident from dramatic reductions in fruit quality attributes. Hence, in future studies a broader analysis of the flow and ultrastructure of phloem sieve tubes is needed.

Funding Support: WAC

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Rootstock/Irrigation Interactions and Their Effect on Vine Water Status, Yield, and Wine Quality Parameters

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With increasing competition for water in Missouri between field crops and anthropogenic needs, it is necessary to find ways to minimize vineyard water use while maintaining wine quality and fruit yield. To investigate rootstock-irrigation interactions a vineyard was established in 2008 in Mt. Vernon Missouri, in a full-factorial experiment varying rootstock and irrigation. 1103P, SO4, 3309C, and self-rooted vines of Chambourcin were planted; irrigation treatments were either (1) unirrigated, (2) full replacement of evapotranspiration (ET), or (3) irrigated at 70% of the potential ET (RDI). Soil moisture, fruit development, canopy density, and yield metrics were monitored with berry samples taken from veraison to harvest and harvested fruit vinified. In the unirrigated and RDI treatment, the drought decreased mean volumetric soil water content. Mean volumetric soil water content differed significantly among treatments for all the rootstocks. Positive correlations were found between midday leaf water potential and the mean volumetric soil water content.

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Midday leaf water potential values during the week before harvest indicated varying tolerance of water stress, with own-rooted vines having higher stress for all three different irrigation regimes than vines grafted to SO4 rootstock. The nonirrigated treatment significantly affected canopy development by reducing the percent of interior clusters and increasing cluster flux availability and flux symmetry. Treatments were found to affect fruit parameters at harvest with differences in berry weight, with SO4 full irrigation producing larger berries than 3309C fully irrigated and self-rooted vines with no irrigation. Additional differences in fruit composition were observed, with higher titratable acidity in fruit from SO4-grafted vines with RDI and nonirrigated treatments and lower pH in nonirrigated, self-rooted vines.

Funding Support: Grape and Wine Institute (ICCVE)

Surface Renewal Detects Changes in Daily Water Requirements of Vines Subjected to Contrasting Irrigation Practices

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Deficit irrigation has evolved as a tool to reduce water use in viticulture because of increasing water scarcity in many agricultural regions. The accomplishment of this objective depends on the accurate knowledge of both vineyard water requirements and vine water status. The improved surface renewal method (SR) is a bio-meteorological technique that can be used to accurately measure crop evapotranspiration (ET_c) at a vineyard scale. A deficit irrigation experiment was carried out in a commercial vineyard of the North Coast Viticultural Region of California. The objective was to determine the impact of irrigation practices on the relationships of SR estimates of ET_c and vine water status parameters, such as leaf water potential (Ψ_{LEAF}) and stomatal conductance (g_s). Three irrigation treatments were applied from veraison to harvest: wet control: vines were irrigated at 100% ET_c ; medium-wet: vines were irrigated between 70 and 80% ET_c ; and moderate deficit: vines were irrigated between 40 and 50% ET_c . SR provided with reliable estimates of daily ET_c compared to the eddy covariance, which is regarded as the reference bio-meteorological method for measuring ET_c . Although Ψ_{LEAF} was consistently higher in the wet control than in medium-wet and moderate deficit treatments, the medium-wet block showed the highest ET_c and g_s values. Surprisingly, vines from the wet and the moderate deficit treatments often exhibited similar ET_c values, regardless of large differences in applied water and Ψ_{LEAF} . A quadratic relationship between ET_c/ET_o and Ψ_{LEAF} indicated that the maximum water demand was reached at mild levels of water stress (~ 0.9 MPa). Conversely,

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vines under wet (>-0.7 MPa) or severe water stress (<-1.4 MPa) conditions showed similar ET_c/ET_0 values. These results showed that the SR technique can be used to better understand the extent of the effect of irrigation practices on both vineyard water use and vine water status in winegrape production.

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Interactive Effects of Mechanical Leaf Removal and Regulated Deficit Irrigation on Merlot Grapevines in Warm Climate

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The goal of this study was to improve the phenolic composition of Merlot x Freedom grown in northern San Joaquin Valley by investigating effects of phenology-based leaf removal timing and regulated deficit irrigation without adversely affecting yield. Three leaf removal and two regulated deficit irrigation treatments were arranged in a factorial split-plot design with four replicated blocks. The leaf removal treatments included no leaf removal (control), pre-bloom leaf removal (PBLR), and three-time postbloom leaf removal (PBBLR) with the goal of intercepting ~20% photosynthetically active radiation (PAR) in the fruiting zone. The two irrigation treatments included a control treatment of sustained deficit irrigation (SDI), where midday leaf water potential (Ψ) was maintained at -1.2 MPa from fruit set to end of harvest with 80% of ET_0 replaced based on weekly K_c measurement, and a regulated deficit irrigation (RDI) treatment maintained Ψ at -1.2 MPa between budbreak and fruit set at 80% ET_0 , Ψ at -1.4 MPa between fruit set and veraison at 50% ET_0 based on weekly K_c measurements, but not thereafter. The PBLR treatment maintained target PAR interception throughout the season. There was no effect of leaf removal or irrigation on fruit set. However, berry and cluster mass was reduced with leaf removal while yield per vine was unaffected. PBLR and RDI increased Brix at harvest. PBLR increased flavonoids, flavan-3-ols, anthocyanins, and tannins in the skin by 30%, and flavanols in the seed at harvest when compared to control or PBBLR. Irrigation treatments did not affect phenolics at harvest. PBLR reduced pruning weight by 23% while Ravaz index and leaf area:fruit increased by 23%, and 16%, respectively. This study provides fundamental information to growers, as the cost to produce a unit of anthocyanin per hectare was reduced by 23% without adversely affecting yield using the above methods.

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Dynamic Changes in Petiole Hydraulic Conductance in Grapevine Leaves Acclimated to Different Light and Water Regimes

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Xylem hydraulic conductance, and therefore water transport, is affected by environmental factors which in turn limit leaf gas exchanges and ultimately plant growth and yield. In this study, we assessed the combined effects of light and water supply received during the growing season on leaf water potential, stomatal conductance, and petiole hydraulic conductance (k_{petiole}) of field-grown grapevines cv. Chasselas at the Agroscope Research Station in Leytron, Switzerland. We tested the hypothesis that petiole hydraulic conductance is more sensitive to water deficits in leaves grown under high radiation than those grown under low radiation. During season 2013, combinations of two irrigation and four radiation levels were studied on Chasselas vines trained in a Pergola system. Irrigated vines were watered weekly with 16 L from bloom to veraison. Nonirrigated vines did not receive any irrigation during the whole season and the soil around them was covered with a plastic film to avoid infiltration from occasional rainfalls. Different leaf radiation levels were obtained by choosing different leaf positions on the Pergola: east, top, west, and interior shaded leaves received 35.7, 61.2, 33.6, and 1.48 mol m⁻² d⁻¹ of photosynthetically active radiation, respectively, measured on day of year 242. Water stress severely reduced leaf water potential, stomatal conductance, and petiole hydraulic conductance along the season. In a diurnal basis, k_{petiole} decreased at midday, especially in irrigated vines, as vapor pressure deficit and evapotranspiration increased and recovered again during the afternoon, while no difference was observed between radiation levels. At the end of the season, at both irrigation levels interior shaded leaves developed larger petioles with higher k_{petiole} than those grown under other radiation levels. This response suggests that not only plant water status but also the radiation regime affected petiole xylem morphology (e.g., number and size of vessels) and therefore k_{petiole} .

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Effect of Preharvest Irrigation Cutoff on Grape Composition and Wine Quality of *Vitis vinifera* L. cv. Malbec

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Irrigation cutoff a few weeks before harvest is a common practice in irrigated viticulture. It is believed that if irrigation is applied until harvest, then grape juice concentration and wine quality will decrease. To test the hypothesis that irrigation cutoff at some time before harvest will concentrate grape compounds and improve wine quality, an experiment was initiated in 2013 in a drip-irrigated Malbec vineyard in Maipú, Mendoza, Argentina. Three cutoff irrigation treatments were applied: Cutoff0, in which vines were irrigated until harvest, and Cutoff15 and Cutoff30 in which irrigation was stopped 15 and 30 days before the estimated harvest date, respectively. Twice a week, leaf net photosynthesis, midday leaf water potential, Brix, acidity, pH, berry weight, and berry volume were measured. Vines were harvested when berries reached 24 Brix and wines from each treatment were made. Total anthocyanin concentration, tannins, and color by CIELab were measured in the wines, and sensory analysis was performed by a trained panel. The Cutoff30 vines presented the lowest leaf photosynthetic rate and water potential, followed by the Cutoff15 and Cutoff0 vines. No differences were found in Brix, acidity, pH, berry weight, berry volume, and yield. In spite of the strong decrease in leaf water potential and photosynthesis due to the preharvest irrigation cutoff, yield components and berry composition were not affected. Wine color and total anthocyanins concentration in Cutoff0 were lower and its color lightness was higher than the other two wines. No differences among treatments were found in aroma, astringency, and bitterness.

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**The Phenomenon of Cavitation in Grapevine (*Vitis vinifera*):
Unraveling Implicated Mechanisms**

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Cavitation is a physiological dysfunction of the xylem of water-stressed plants that leads to loss of hydraulic conductance. This negatively impacts water supply, affecting leaf water potential. Stomatal closure is an effective response upon diminishing foliar hydraulic contents. Depending on the species, stomata closure can prevent catastrophic cavitations. This research intended to understand how stomatal control acts upon cavitation events in two contrasting grapevine (*Vitis vinifera* L.) varieties, Grenache and Syrah. We hypothesized that water-stressed grapevines reduce stomatal conductance, avoiding cata-

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strophic embolism, and that some varieties, when grown under water stress, may acclimate by developing a precise stomatal control or a less vulnerable xylem. A randomized experimental plot inside a greenhouse was conducted to test two water-stress treatments. Gas exchange measurements and stem water potential were assessed hourly from predawn to 16 hr. Subsequently, cavitation curves were constructed, and the level of embolism reached during the day as well as plant hydraulic conductance were calculated. Finally, we determined the relationship among stomatal conductance, hydraulic conductance, and embolism. By means of a mechanistical model, based on the water and vapor fluxes, hydraulic conductance, stomatal conductance, and the vulnerability to cavitation, we probed that stomatal conductance is not the only variable that controls cavitation. We determined that it is coupled with the hydraulic conductance, and this coupling (i.e., the ratio between stomatal and hydraulic conductances) is responsible for achieving embolism control. We concluded that grapevines under mild water stress do not need to close their stomata in order to avoid cavitation, therefore, no cost of carbon assimilation occurs.

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Application of a Prototype System for Irrigation Scheduling Based on Satellite Mapping of Water Requirements in California

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Satellite mapping of evapotranspiration (ET) from irrigated agricultural lands can provide water managers and agricultural producers with information that can be used to optimize agricultural water use, especially in regions with limited water supplies. In particular, the timely delivery of information on agricultural crop water requirements has the potential to make irrigation scheduling more practical, convenient, and accurate. We present findings from the development and deployment of a prototype system for irrigation scheduling and management support in California. The Satellite Irrigation Management Support (SIMS) framework utilizes the NASA Terrestrial Observation and Prediction System to map crop canopy development and basal crop coefficients (K_{cb}) for multiple crop types in the Central Valley of California at the scale of individual fields. SIMS data is then integrated with reference evapotranspiration (ET_0) from the California Irrigation Management Information System (CIMIS) to map basal crop evapotranspiration (ET_{cb}). The GIS and remote sensing group at E&J Gallo is also implementing a system based on satellite imagery from the Landsat platform (USGS) to assist with irrigation scheduling across different growing regions. Information from these systems is distributed to farm managers and irrigators via a web-based irrigation management decision support system and web services. We present the prototype systems and describe joint

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efforts to apply these data frameworks developed by NASA and E&J Gallo. We also present comparisons of estimates of crop water needs from the prototype system against estimates of ET from other methods, including eddy covariance, surface renewal/energy balance stations, and observations from wireless soil moisture sensor networks deployed in vineyards in California.

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Limitations to Photosynthesis during Water Deficit in Three Grapevine Cultivars

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Low water availability (i.e., water deficit) is one of the major factors affecting plant growth and yield in many crops worldwide. The effects of water deficit on growth and yield are linked to carbon assimilation. The main limitation imposed by water deficit on photosynthesis is a limited CO₂ diffusion mediated by stomata closure. However, a decrease in mesophyll conductance, electron transport rate through photosystem II, or a relative increase in photorespiration have also been observed in different studies. The degree of importance of these processes to photosynthesis limitation under water deficit may be different depending on its intensity and on the cultivar-specific response. In this study, we analyzed the effect of water deficit on different mechanisms (e.g., stomata closure, mesophyll diffusion, biochemistry) that limit photosynthesis in three grapevine cultivars. For this aim, we established an experiment in Mendoza, Argentina, in one-year-old potted grapevines of Syrah, Malbec, and Bonarda. Irrigation treatments consisted of fully irrigated vines (100% of their daily consumption) and deficit-irrigated vines, which were submitted to progressive drought by withholding irrigation during a month. We measured leaf water potential and soil water content. Combined measurements of chlorophyll fluorescence and gas exchanges were used to calculate mesophyll conductance, efficiency of photosystem II, electron transport rate, and non-photochemical quenching. Finally, we performed a quantitative limitation analysis in order to evaluate the different limitations to photosynthesis. Our results showed a steep decrease in leaf water potential and soil water content after a month of water deficit. At the beginning, stomatal conductance was rapidly affected, whereas mesophyll conductance and photosynthesis were only moderately regulated. Only when drought became severe, mesophyll conductance, efficiency of photosystem II, and electron transport rate were highly affected. Our findings showed that under severe water stress, diffusion within the mesophyll and biochemical impairment limited photosynthesis.

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Occupational Competencies for the Grape and Wine Industry: Stakeholders Know Best

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The Viticulture and Enology Science and Technology Alliance (VESTA) is funded by the National Science Foundation and is based on the premise that stakeholders know best when it comes to developing and implementing online courses, field experiences, and professional development workshops that address the workforce needs of the grape and wine industry. Accordingly, industry representatives, extension agents, educators, and students serve on state level advisory committees and participate in annual reviews of the curriculum. VESTA learned from stakeholders that while some vineyards and wineries have internally developed expectations for their workforce, only New Zealand has defined standards for tasks that are applied across its industry. This is in sharp contrast to many U.S. industries that have established competencies and/or standards for their workforce. Therefore, VESTA initiated a research project to develop occupational competencies that will enable employers to refine their hiring practices and educators to enhance their curricula to better prepare future employees. Eighteen positions were identified and matched with existing occupations in the U.S. Department of Labor's Standard Occupational Classification System. Led by recognized experts in viticulture and enology, the essential characteristics of the occupation and the worker were defined for each of these occupations, and then reviewed by stakeholders throughout the 20 states with institutional members in VESTA. A formal review of the draft occupational competencies was conducted in December 2013 by stakeholders selected to provide a broad representation of the industry and academia throughout the country. Of the 26 participants who responded to a post-conference survey, 100% reported that the results will be useful for defining industry jobs, 92% indicated they will be used to develop job descriptions used in hiring, and 92% reported they will contribute to the alignment of enology, viticulture, and related educational programs with industry needs.

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