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SIXTYFOURTH NATIONAL CONFERENCE

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TECHNICAL ABSTRACTS

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Oral Presentation Abstracts

Wednesday, June 26

Thursday, June 27

Poster Presentation Abstracts

Wednesday, June 26 and Thursday, June 27

NOTE: pp. 1-56 constituted the program section of the original printed *Program and Technical Abstracts* booklet.

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

Abstracts are those submitted and accepted through the 2013 Call for Abstracts and presented at the 2013 National Conference.

2013 National Conference Technical Abstracts

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Alphabetical Listing of Presenting Authors

Enology — Flavor/Analysis Session

Evolution of Oak Lactone from Glycoconjugate Precursors during Toasting and Wine Maturation

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Oak maturation plays an important role in the production of high-quality wine, enhancing both physical attributes (color and stability) and sensory properties (aroma, flavor, and astringency). Of the 200 oak-derived volatile compounds identified in oak-aged wine and spirits to date, the most important are considered to be the *cis*- and *trans*-isomers of oak lactone, which contribute woody, citrus, vanilla, and coconut aromas. Oak lactone is a natural component of oak wood, but it also exists in glycoconjugate precursor forms. This study concerned the role of glycoconjugates of 3-methyl-4-hydroxyoctanoic acid, specifically a galloylglucoside, glucoside and rutinoside, in the evolution of oak lactone in wine during oak maturation. The glycoconjugate profiles of 10 French oak samples were obtained by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using stable isotope dilution analysis. The galloylglucoside was found to be the predominant glycoconjugate precursor and ranged in concentration from 110 to 354 µg/g. Maturation trials were then conducted to investigate the accumulation of oak lactone and hydrolysis of glycoconjugate precursors in model wine over time. It was reported that the galloylglucoside undergoes acid-catalyzed hydrolysis after extraction into wine; after 12 months maturation, the glucoside was the most abundant precursor, present at between two- and eleven-fold higher concentrations than those observed for powdered oak. The effect of toasting on the glycoconjugate content of oak was also investigated. Thermal degradation of glycoconjugates was only observed when oak samples were heated at 200°C for 30 minutes, demonstrating their thermal stability. As with free oak lactone, the glycoconjugate content of oak wood showed considerable variability. Future work could therefore investigate the influence of factors such as geographic origin, species, and seasoning on the glycoconjugate profiles of both French and American oak.

Funding support: AWRI, Australia; Tonnellerie Seguin Moreau, France

The Genetic Basis of Methoxypyrazine Production in Grape Berries

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The key to manipulating and predicting grape berry composition to meet specific wine outcomes lies in the understanding of the biochemistry behind the production of grape metabolites. Methoxypyrazines are a family of potent grape-derived volatile compounds that impart green/herbaceous flavors to

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Enology — Flavor/Analysis Session – CONTINUED

wines of certain varieties, including Cabernet Sauvignon. The concentration of the most abundant methoxypyrazine, 3-isobutyl-2-methoxypyrazine (IBMP), in grapes at harvest can be highly variable, as IBMP accumulation is influenced by many viticultural and environmental factors. Unfortunately, the biochemistry behind the production of the methoxypyrazines is not clear. To address this issue, the fact that the Pinot Meunier dwarf mutant does not produce methoxypyrazines was used to produce genetic populations which segregate for the production of IBMP in berries. This allowed the mapping of this trait to a small region of the genome. Subsequently, a candidate gene was identified in this genomic region, which can catalyze the last step in the production of IBMP in berries.

Funding support: Grape and Wine Research and Development Corporation, Flinders University of South Australia, and Commonwealth Scientific and Industrial Research Organisation (CSIRO)

A Comparison of Extraction Techniques for GC-MS/MS Analysis of Odor-Active Pyrazines in Wines

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Methoxypyrazines are volatile compounds produced in grapes that impart vegetal characters to some wines (such as Sauvignon blanc and Cabernet Sauvignon). Multicolored Asian ladybugs present on grapevines that then get incorporated into the winemaking process can also be a source of methoxypyrazine taint. High levels (>10 ng/L) of methoxypyrazines are associated with consumer rejection in wine; therefore, sensitive analytical methods that quantify methoxypyrazines at sensory threshold concentrations (<2 ng/L for 3-isobutyl-2-methoxypyrazine [IBMP] and 3-isopropyl-2-methoxypyrazine [IPMP]) or below are needed. Previous methods to analyze methoxypyrazine are laborious, time-consuming, not automated, not sensitive enough, or may suffer from matrix interferences. This work quantifies levels of four methoxypyrazines commonly found in wines—IBMP, IPMP, 3-sec-butyl-2-methoxypyrazine (SBMP), and 2-methoxy-3-ethylpyrazine (MEP)—using deuterated internal standards. We present a comparison of two extraction method for analysis of these analytes: head-space solid-phase microextraction (HS-SPME) and stir-bar sorptive extraction (SBSE). These extraction methods were then combined with gas chromatography-tandem mass spectrometry (GC-MS/MS) using positive chemical ionization (PCI). Both methods of extraction, when coupled to GC-MS/MS, require little sample preparation, are high throughput, and accurately measure levels of methoxypyrazines below reported sensory thresholds.

Funding support: not funded

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Enology — Flavor/Analysis Session – CONTINUED

High-Throughput MS Analysis for Characterizing the Phenolic Composition and Color of Rosé Wines from around the World

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Color is essential in the assessment of rosé wine quality. Rosé wine pigments include anthocyanins extracted from the grapes and derived pigments formed from them during winemaking. In a collaborative study carried out by Centre du Rosé, Institut Français de la Vigne et du VIN (IFV), and INRA Joint Research Unit Sciences for Enology (UMR Sciences pour l'Oenologie), 285 rosé wines from 22 wineproducing countries worldwide were characterized. A method based on ultra-high-resolution liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) has been developed for fingerprinting the phenolic composition. Using a high throughput and highly sensitive method, multiple reaction monitoring (MRM) mode, that enables selective quantification of target compounds from their characteristic mass fragments, 125 phenolic compounds have been quantified in the 285 wines. To correlate this large data set with origin, color data, and enological parameters (including alcohol, sulfites, and pH), chemometrics using multivariate analysis was performed. A great diversity of color and composition was shown. The resulting profiles and associated color characteristics can be related to product origin and enological practices. In particular, extraction and oxidation were found to have a major impact in the determination of pigment composition, color, and final wine style, probably related to marketing targets.

Funding support: Centre du Rosé, Institut Français de la Vigne et du Vin, INRA, UMR 1083 SPO-Plateforme Polyphenols

Smoke Taint in Grapes and Wine: What's the Risk and Can It Be Managed?

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While wildfires are a relatively common event, their impact on the grape and wine industry has only recently been considered a major threat. Widespread fires in southeast Australia in 2003, 2006, 2009, and 2013 and in California in 2008 have heavily impacted the wine industry as a result of smoke contamination of the fruit, often referred to as smoke taint. The perception of smoke taint is due to the presence of volatile phenolics, such as guaiacol, cresol, and syringol and their derivatives in the wine. These compounds are lignin

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degradation products common in smoke and are found in grapes and wine following smoke exposure of the fruit. Initially only guaiacol and 4-methylguaiacol were considered important and used as indicators in diagnostic testing. However, 4-methylguaiacol has not been observed above its odor threshold in grapes or wine, and numerous other compounds have now been identified with confirmed odor activity. Early detection methods analyzed only free phenols. However, observations of guaiacol increasing over time have led to speculation and subsequent discovery of a pool of precursor compounds. These compounds are glycosidic conjugates of the phenols and over time the glucosides are cleaved in the wine releasing the volatile phenols, which increases taint in the wine. Current investigations through the Centre for Expertise in Smoke Taint Research are examining the mode of entry on smoke taint compounds into the fruit, the evolution of smoke taint in wine over time with a view to predicting shelf life, varietal differences in accumulation, and strategies to manage the risk posed by smoke from wildfires and controlled burning.

Funding support: The Centre for Expertise in Smoke Taint Research (CESTR), a national collaborative project between the Department of Primary Industries, the Australian Wine Research Institute, and the Western Australian Department of Agriculture and Food

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Viticulture — Rootstocks Session

The Origin of *Vitis doaniana*, a Wild Grapevine Species with Excellent Chloride Exclusion Capability

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Wild *Vitis* species from the southwest Untied States are a promising resource for rootstock breeding. Recently, we identified several populations that restrict chloride uptake from the roots to the shoots, making them good candidates for breeding salt-tolerant rootstocks. However, because wild grapevines readily hybridize across species and can be transported long distances by birds, we cannot efficiently use them for breeding until we better understand the complex genetic relationships between these species. One such group with excellent chloride exclusion capacity, but an uncertain genetic background, is *Vitis doaniana* from the Red River region between Texas and Oklahoma. In 1909, T.V. Munson first described this species and suggested that it may be a hybrid of *V. acerifolia*, found in southern Oklahoma and northern Texas, and *V. candicans*, found throughout Texas. To test the theory of a hybrid origin, we extracted DNA from several of our collections from this region and genotyped them with a large set of microsatellite markers. Our results indicate that *V. doaniana*, found only in a region where both *V. acerifolia* and *V. candicans* also grow, is likely a hybrid of these two species. We will present a detailed genetic analysis of the vines from this region, coupled with breeding traits such as the chloride exclusion capacity and dormant rooting ability. We believe that as the costs of genotyping drop, analyses of this kind will be an increasingly important component of rootstock breeding and lead to more informed decision making at the earliest stages of breeding programs.

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

Application of Two Drought Avoidance Assays in *Vitis* Rootstocks

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Highly divergent patterns of root architecture have been described in grape rootstocks, ranging from shallow, fine roots seen in Riparia Gloire (*Vitis riparia*) to deep, thick roots in Ramsey (*V. champinii*). Although many physiological factors contribute to drought resistance, these simple categories of root growth may be a major component and may also help to explain differences in scion

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Viticulture — Rootstocks Session – CONTINUED

vigor under stressed conditions. Because an analysis of root architecture can be resource intensive and therefore not useful in a high-throughput breeding program, we previously compared a variety of methods to optimize root architecture measurements. Deep rhizotron containers produced excellent developmental insight and were useful for comparing tens of genotypes. Rooting angles of adventitious roots from herbaceous cuttings were quickly recorded and appeared to provide sufficient data quality for characterizing large populations. Our first application of these methods was to characterize the root architecture of the entire set of widely-available rootstocks currently grown in California, using the rhizotron container method. A rapid proliferation of deep roots at the expense of root growth near the surface was noted in some genotypes generally regarded as drought resistant: 1103P, Ramsey, and St. George. Salt resistant genotypes characterized in other studies were not associated with a single rooting pattern, implicating xylem parenchyma transporters as the major variable in chloride exclusion rather than an architecture-based avoidance of saline soil layers. Some rootstocks and new, experimental genotypes were very slow growing, to such a degree that comparisons with standard rootstock biocontrols were not possible. These genotypes will require an additional assay that incorporates a longer establishment period. Multiple hybrid populations are currently being tested for rooting angle in the adventitious root assay, with the intent of molecular marker generation from an appropriately segregating population.

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

Evaluation of New Grape Rootstocks Resistant to Abiotic Stresses

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The objective of the present research was to investigate the performance of four new rootstocks resistant to different abiotic stresses (drought, salinity, and heat) and obtained after a long breeding program. The four rootstocks—M1, M2, M3, and M4—were studied under field conditions and compared to the most widely used ones, including 140 Ru, 110R, 1103P, SO4, 420 A, and 41B, according to a two-year trial (2011–2012). In order to evaluate rootstock adaptability to different environmental conditions, experimental vineyards were established in several sites within the Italian territory, characterized by different climate and soils. Autochthonous and international grape varieties,

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including Corvina, Cabernet Sauvignon, Uva di Troia, and Magliocco, were grafted onto each of the new rootstocks to evaluate the scion-rootsctock interaction. The main vegetative and productive parameters were evaluated (number of buds per vine, number of shoots per vine, number of bunches per vine, yield per vine, pruning weight per vine) along with the grape composition (soluble solids, titratable acidity, pH). The rootstock/soil interaction was studied in deep, by analyzing the root density and distribution. For each rootstock, 1 m deep trenches were dug at 40 cm and 100 cm from the vine row and roots were counted and divided in different diameter classes. The observed differences confirmed that the root system distribution and density depend on the rootstock, on the soil water availability, and on the soil physical characteristics. In dry environments and sandy soils, M3 highlights a higher ability to explore the soil vertical and horizontal profile compared to the other rootstocks.

Funding support: Progetto AGER- SERRES, grant 2010-2105

Investigating the Molecular Basis of Grafted Grapevine Adaptation to Water Deficit

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A multidisciplinary approach (ecophysiology/genomic/genetic) that uses the most recent facilities and technologies was undertaken for a comprehensive analysis of grapevine response and adaptation to water-limiting conditions. Using a phenotyping platform that allows the progressive application of well-defined levels of water deficit, drought experiments have been conducted with four rootstock/scion combinations between the two *Vitis vinifera* varieties Syrah and Grenache and the two rootstocks Riparia Gloire de Montpellier and 110R, exhibiting contrasted sensibility and response to water deficit. Fine ecophysiological measurements have been performed and biological samples from root and scion have been harvested and subjected to global transcriptome analyses using Nimblegen grape whole-genome expression arrays. In parallel, phenotyping experiments have been conducted in control and water deficit conditions with an interspecific Cabernet Sauvignon x Riparia Gloire de Montpellier progeny used as rootstock and Cabernet Sauvignon as a single scion to identify zones in the genome which control the different responses to water deficit. Finally, and because large data sets are generated by this project, we have created a dedicated database called Vit-Phe to associate transcript expression levels with ecophysiological and physiological data. We are now in the process of analyzing these important data sets to understand the phenotypic plasticity of grapevine in response to water deficit and to identify candidate

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Viticulture — Rootstocks Session – CONTINUED

genes involved in the root response to water deficit. The results of this study will contribute to the identification of molecular markers of drought tolerance that might be used for both rootstock and scion selection and improvement.

Funding support: Aquitaine Council Region Conseil Interprofessionnel des Vins de Bordeaux National French Agency French National Committee of Wines to AOC Inter-trade

Yield and Wine Sensory Properties of Chardonnay and Shiraz from Rootstocks Differing in Capacity for Salt Exclusion

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Yields were determined and wines were made in 2011 (Chardonnay) and 2012 (Shiraz) from grapes harvested from vines on own roots (Chardonnay) or from Chardonnay and Shiraz on a range of rootstocks differing in capacity for chloride and sodium exclusion. The wines were evaluated using sensory descriptive analysis. Chemical composition was also determined. Irrigation water electrical conductivity was 1510 and 1100 µS/cm for Chardonnay and Shiraz, respectively. The rootstocks were 140 Ruggeri, K 51-40, Kober 5BB, 1202C, and Schwarzmann (Chardonnay) and 140 Ruggeri, 1103 Paulsen, 110 Richter, Ramsey, 101-14, Merbein 5489, Merbein 5512, and Merbein 6262 (Shiraz). For Chardonnay, the K 51-40 wines were rated highly for the attributes salty and viscosity and relatively low in acidity. Mean wine chloride and sodium concentrations in these wines were 407 and 374 mg/L, respectively. Potassium concentration did not relate significantly to any sensory attribute. Wines from K 51-40 and Schwarzmann rootstocks also received high scores for fruit attributes. The mean concentration of chloride in wines from K 51-40 was higher than the sensory detection threshold determined for sodium chloride in white wine. For Shiraz, mean chloride, sodium, and potassium concentrations in Merbein 6262 wines were 482, 26, and 1344 mg/L, respectively. Chloride and potassium concentrations in these wines were significantly higher than in wines from the other rootstocks (range 96–172 mg/L for chloride, and 913–1076 mg/L for potassium). Sodium concentrations in all these wines were low (range 26–37 mg/L). The sensory effect of the relatively high potassium and chloride levels in wines from this rootstock in comparison to the other wines will be discussed.

Funding support: Grape and Wine Research and Development Corporation, Australia

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Enology — Micro/Molecular Biology Session

A Metabolomics Approach to Study Chardonnay Fermentations

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The transformation of grape juice to wine is a complex metabolic relationship between two species, *Vitis vinifera* and *Saccharomyces cerevisiae*. Grape juice, composed primarily of water, sugar, organic acids, and additional secondary metabolites, provides nutrients for the yeast resulting in the production of wine, composed primarily of water, ethanol, glycerol, organic acids, and additional components. The final molecular composition, developed from the grape/yeast relationship, contributes to the flavor, aroma, and mouthfeel of the wine. In this study we examined this complex relationship by identifying the exo- and endometabolome at three time points of a Chardonnay wine fermentation. We identified 227 metabolites in the exometabolome and 404 metabolites in the endometabolome, each of which was placed into metabolic pathways or families. Considerable metabolic variation was seen at each time point, allowing us to describe patterns of primary and secondary metabolism during fermentation. Our results suggest that the regulation of metabolic pathways is coupled to fermentation progress. These data provide an understanding of the differential utilization and production of primary and secondary metabolites during a wine fermentation. This work provides key understanding of cell communication mechanisms, metabolic engineering, and industrial biotechnological processes.

Funding support: E. & J. Gallo Winery

A Novel *Saccharomyces cerevisiae* Strain Tracking Method for Quantifying Strain Mixtures during a Wine Fermentation

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Strains of *Saccharomyces cerevisiae* in wine fermentations can be diverse, even when tanks are inoculated with a specific strain. In a previous study, it was found at several Canadian wineries that numerous commercial strains were responsible for the fermentation of Pinot noir wine. Furthermore, a specific commercial strain was consistently found to coferment with another commercial strain. The aims of this study were to develop a visual method to quantify multiple strains throughout a fermentation and to test its accuracy by comparing it with a commonly used molecular method in an experiment designed to observe the interaction of two different strains (Lalvin RC212 and Lalvin D254). Blue (495 nm emission) and red (631 nm emission) highly fluorescent nanoparticles, quantum dots (QDs), were conjugated with

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Enology — Micro/Molecular Biology Session – CONTINUED

glutathione and incubated with different yeast strains prior to co-inoculation. Yeast cells contained either blue or red QDs and were quantified based on QD color using confocal microscopy. To determine the competitive interaction of strains under laboratory conditions, fermentations were conducted in sterile Pinot noir must at different inoculation ratios. Data were quantified by either using a microsatellite method or by using the quantum dot method described above. In both methods, commercial strains remained at the initial inoculated ratios throughout fermentation. Quantification from both the quantum dot and the microsatellite methods were strongly correlated. We found that both QD-labeled yeast strains were distinguishable throughout the entire fermentation based solely on QD color. Moreover, QD-labeled glutathione was successfully internalized and transferred at detectable levels to daughter cells, which allowed for the quantification of the entire yeast population. Compared to traditional microsatellite techniques, our QD method allowed us to analyze 50x more cells throughout fermentation at less than half the cost of microsatellite analysis.

Funding support: Natural Sciences and Engineering Research Council of Canada, Quails Gate Estate Winery

Tracking Microbial Terroir Along the Grape-to-Glass Continuum

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On their journey from the vineyard to the wine bottle, grapes are transformed to wine through microbial activity, with indisputable consequences for wine quality parameters. However, the determinants of regional wine characteristics are most commonly assumed to stem from viticultural end environmental factors influencing grape development. We propose that these same factors may shape grape-surface microbiota, which, in turn, directly and/or indirectly influence wine fermentations. Using high-throughput sequencing analysis of grape, wine, and winery surface microbiota, we have tracked the shifting microbiome that accompanies wine on this journey, demonstrating that unique microbial communities create regional and variety-specific signatures. Additionally, the winery environment is an important reservoir for two-way transfer of microbes between wine fermentations. The combination of regional origin, varietal background, and winery surface interactions shapes the unique, traceable microbial populations of wine fermentations, with strong implications for product quality.

Funding support: Wine Spectator Scholarship (for NAB)

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Enology — Micro/Molecular Biology Session – CONTINUED

The Impact of Different Processing Steps on Yeast Biodiversity of Spontaneous Fermented Riesling and Pinot noir Musts

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Spontaneous fermentations have a great renaissance and are popular for many winemakers to produce wines with higher individuality in contrast to more or less uniform wines inoculated with starters. But spontaneous fermentations could have very often a negative influence on the aroma of the resulting wines. It is well known that not only the yeast flora on the grapes but also yeasts on the surface of the equipment of a winery have a great impact on spontaneous fermentations. But less data are available about the influence of different enological grape processing methods and their impact on spontaneous fermentations of white and red wines. The investigation of these topics, the influence of several grape processing methods on populations of yeasts and later on the spontaneous fermentations during Riesling and Pinot noir wine production, were part of a project supported by the FEI/AiF. For the investigation of the influence on yeast populations and aromas by the processing of white grapes, ground Riesling grapes were compared to approaches with 6 hr and 24 hr of maceration of the same variety. Pinot noir grapes were used to examine the effects on yeast biodiversity and aroma using different processing methods of fermentation such as mash heating, mash fermentation, and a combination of cold maceration followed by mash fermentation. Sample material was taken at different time points during processing and fermentation to isolate and identify occurring yeasts and to analyze typical compounds. For each sampling point, 100 randomly isolated yeasts were identified by FTIR spectroscopy. The wines produced by different processing methods were tasted and described sensorial by a tasting panel. All data were the basis to correlate the different grape processing methods to the occurring yeast populations and the aromas of the spontaneous fermented wines.

Funding support: FEI/AiF

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Enology — Micro/Molecular Biology Session – CONTINUED

Use of Metabolomics Analysis to Identify Growth Substrates of *Brettanomyces bruxellensis* in Wine

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Brettanomyces are a major spoilage problem in wine and other beverages, particularly during storage. The growth of *Brettanomyces* in wine is often associated with nutrient availability, strain, storage conditions, and physical/chemical properties of the wine. The metabolic functioning of *Brettanomyces* in wine is remarkable given the stressful conditions, but the substrates supporting establishment, growth, and metabolism of this organism remain largely unknown. In order to understand the metabolism of *Brettanomyces* in wine, we conducted a comprehensive metabolomic analysis of the metabolic pathways induced and active in different *Brettanomyces* strains growing in wine. This analysis identified several potential growth and energy supporting substrates that were taken up by *Brettanomyces* from wine immediately upon introduction. Based on availability and activity, we compiled a list of over 50 metabolites that were subsequently tested for their ability to support the growth of *Brettanomyces* as a carbon and energy source. Five diverse strains were tested to determine the ability of members of this genus to utilize a spectrum of carbon sources. The genome of one of the strains being tested has been fully sequenced, allowing for future investigations into the metabolic pathways and their genetic counterparts. Preliminary results indicate that several *Brettanomyces* strains are capable of utilizing citric acid cycle intermediates as sole carbon sources under aerobic conditions. This is a significant finding as these acids can be found in wine following the alcoholic fermentation and growth of *Brettanomyces* is stimulated by the availability of oxygen. Further investigation into other compounds involved in this cycle could help elucidate the pathways and enzymes involved and their role in establishment of *Brettanomyces* infections of wine. Also under investigation are breakdown products of ascorbic acid as well as phenolic compounds as several strains showed a tendency to absorb these components from the wine.

Funding support: UC Davis Department of Viticulture and Enology Wine Yeast and Bacteria Culture Collection

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Viticulture — Pests & Diseases Session

Genetic Analysis of Phylloxera across the Eastern and Southwestern United States

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Grape phylloxera have been a destructive force in viticulture since their accidental importation from their native range in America into Europe. The largest segment of their native range spans from Texas north into the Dakotas and east all the way to the Atlantic Ocean. A second smaller range covers the Southwestern states of Arizona, Nevada, and Utah. In an attempt to begin to understand the genetic diversity across this vast range, foliar gall samples were collected from 155 plants from 19 states. An initial look at the data from a single insect sample per plant, run against 32 simple sequence repeat markers, found three distinct genetic groups separated by both geographic range and host. Additional samples collected from each plant are currently being run. Preliminary evaluations of this data have shown that phylloxera collected from the same plant, and even the same leaf, can be genetically distinct. This data will hopefully give insights as to the reproductive mode that phylloxera use across their native range. To ascertain the source of phylloxera in different introduced regions, samples collected in California and samples sent from Hungary, Austria, Uruguay, Brazil, Peru, and Argentina,were also included. These samples have indicated that the New York and Pennsylvania region is the source for most of the phylloxera across these grapegrowing regions.

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

Genetic Resistance to Powdery Mildew in *Vitis rupestris*: Mapping a Way to Generate Durably Resistant Cultivars

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Genetic resistance to the powdery mildew fungus (*Erysiphe necator*) has been characterized from diverse sources. Strong resistance loci such as *Run1* and *Ren1* are being used in grapevine breeding programs, but individually these strong sources of resistance may not be durable. Many North American grapevines that coevolved with powdery mildew developed durable but partial resistance to the pathogen. This partial resistance could be combined with stronger resistance genes to provide long-term resistance. In this work we aimed to characterize natural powdery mildew resistance in *Vitis rupestris* B38

*indicates corresponding author

through microscopy and genetics. First, we quantified the penetration and microcolony success rate of different isolates of powdery mildew on resistant and susceptible grapevines. Powdery mildew isolates that were able to overcome *Run1* resistance were among the least virulent on B38. Second, we analyzed the segregation of powdery mildew resistance in a cross of *V. rupestris* B38 x Chardonnay. We also created dense maps using next generation sequencing and bioinformatics approaches, obtaining significant improvements over the current standards. By using genome-wide association analysis, we were able to map regions of the genome responsible for resistance in *V. rupestris* B38 and susceptibility in Chardonnay. Here we present the first insights into the genetics of *V. vinifera* susceptibility to powdery mildew. Based on our results, B38 could be a valuable source of powdery mildew resistance that may help to preserve the effectiveness of stronger loci such as *Run1*. In this sense, the loci found and the maps generated could be useful to help introgress B38 resistance into a cultivated background, while avoiding regions of the *V. vinifera* genome that confer susceptibility.

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Phylogeographic Analysis of Resistance to Pierce's Disease in North American and Mexican Species

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Pierce's disease (PD) resistance exists in many native grape species from the Gulf Coast states (Florida, Georgia, Mississippi, Alabama, Louisiana, and Texas), leading to speculation that the bacterial causal agent, *Xylella fastidiosa*, has been present for a long period to allow the native grape species to evolve resistance. Many accessions collected from northern Mexico have also shown strong resistance to PD. Most of the accessions from the UC Davis Mexican species collections appear to be introgressive hybrids among *Vitis arizonica*, *V. berlandieri*, *V. candicans* (*V. mustangensis*), *V. cinerea* var. *floridiana*, *V. girdiana*, and *V. monticola*. Strong resistance to PD occurs in *V. arizonica/candicans, V. arizonica/girdiana,* and *V. arizonica/monticola* forms. The goals of this study are to investigate the phylogeographic diversity of plant material collected from Gulf Coast states and northern Mexico with SSR markers, establish distinct genetic groups and identify hybridized species based on the genotypic data, carry out PD resistance evaluation via ELISA, and correlate the PD resistance with defined groups to better understand the evolution of PD resistance. A total of 160 accessions collected from Gulf Coast states and northern Mexico were genotyped with 22 SSR markers selected to cover grape's 19 chromosomes. The genotypic data will be analyzed with hierarchical clustering (Ward

method) and model-based clustering methods implemented in the program STRUCTURE. Novel sources of PD resistance will be discussed in the context of an ongoing breeding program and in relation to their taxonomic relationships.

Funding support: CDFA Pierce's Disease/Glassy-Winged Sharpshooter Board and the Louis P. Martini Endowed Chair for Viticulture

The Costs and Benefits of Pierce's Disease Research in the California Winegrape Industry

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Pierce's disease (PD) of grapevines is endemic to California and represents a significant threat to the California grape industry, which contributed $3.0 billion (8.5%) to the value of California's farm production in 2010. PD is caused by a bacterium that is spread by a group of insects called sharpshooters. We examined potential returns to research aimed at combating Pierce's disease. To do this, we use a simulation model to evaluate likely payoffs from these technologies in a range of scenarios. Our results are derived from an analysis using a regionally disaggregated dynamic simulation model of the California winegrape market, with specific representation of the impacts of PD/glassy-winged sharpshooters (GWSS) on the vineyard stock (vines that last up to 25 years in the absence of disease) and on alternative technologies anticipated to be developed from the past and ongoing research program. We estimate the payoffs from these innovations over a 50-year horizon under various alternative scenarios regarding the status of the PD/GWSS control program and corresponding disease incidence. In one scenario, we assume the control program stays in place over the 50 years and prevents a large-scale PD outbreak (a scenario in which the GWSS would become endemic throughout California). In this scenario, annual average benefits range from $4 million (for a technology that becomes available in 40 years and is adopted by 40% of PD-affected growers) to $56 million (for a technology that is available in 10 years and is adopted by 100% of affected growers). In an alternative scenario, in which the PD control program ends and a large-scale outbreak results, we estimate that the average annual benefits from these technologies would range from $7 million to $126 million over the 50-year horizon.

Funding support: California Department of Food and Agriculture, University of California, Davis Giannini Foundation for Agricultural and Resource Economics

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Viticulture — Pests & Diseases Session – CONTINUED

The Role of Nitrogenous Compounds in the Occurrence of Pinot Leaf Curl

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Pinot leaf curl is a disorder that affects several Pinot varieties grown in areas with cool spring temperatures in coastal valleys. Symptomatic leaves on elongating shoots curl downward across the middle of the lamina, perpendicular to the mid-vein. The angle of the downward bend is acute; the marginal tip of the center lobe may come into contact with the petiole. Mild symptoms involve only the lamina; continued expansion of which results in a misshaped and reduced leaf size. A necrotic region is present on the mid-vein preventing further elongation. If the necrosis expands to include the petiole, the leaf will abscise from the shoot. Severe symptoms occur when necrosis involves the node at which a leaf abscised, killing the shoot distal to that point. Crop load is reduced in severely symptomatic vines. In 14 vineyard blocks in Sonoma County, symptomatic and asymptomatic leaves were sampled in May and June 2012 and concentrations determined in laminae for putrescine (1,4-diaminobutane) and amino acids. The level of putrescine was found to be elevated in symptomatic versus asymptomatic samples in 12 of the 14 pairs ranging from 0.6 to 10 µmoles per gram fresh weight in symptomatic tissue as compared to 0.2 to 6 µmoles per gram fresh weight in asymptomatic tissue. Relative amino acid levels were less clearly aligned with symptoms; however, in six pairs, symptomatic lamina had elevated gluatamine levels in addition to elevated putrescine. Pinot leaf curl appears to be associated with elevated nitrogen levels in laminae. Elevated putrescine levels are toxic to plant tissue and are thought to be involved in symptom development in false potassium deficiency ("spring fever") and early bunch stem necrosis. Pinot leaf curl may be an additional disorder associated with putrescine.

Funding support: American Vineyard Foundation

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Enology — Sensory/Sensory Impacts Session

Unravelling the Nature of Perceived Minerality in White Wine

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The presentation will describe research aimed at delineating the nature of perceived minerality in white wine. To investigate the frequently used but ill-defined descriptive term *mineral* as applied to wine, we conducted sensory and physicochemical analyses on Sauvignon blanc wines from two major Sauvignon-producing countries, New Zealand and France. Sensory experiments were conducted in Marlborough, New Zealand and in three regions of France: Bordeaux, Burgundy, and Sancerre/Loire. The same 16 wines (eight French, eight New Zealand) were characterized organoleptically by a total of 63 wine professionals (31 New Zealanders and 32 French) under three conditions, bouquet only (orthonasal olfaction), palate only (nose-clip condition), and full tasting (orthonasal olfaction, retronasal olfaction, taste, Trigeminal stimulation). Sensory data show that French and New Zealand participants overall evaluated the wines similarly, separating the 16 wines by country of origin, with hedonics (i.e., liking) an important factor underlying the separation. Other key sensory results include demonstration of an association between perception of minerality and perception of sourness/acidity; demonstration of an association between several assumed subcomponents of perceived minerality (e.g., the descriptor lead/graphite) with perceived bitterness and with the descriptor sulfide (i.e., reductive character); and the finding that minerality was perceived under all three modes of sensory evaluation including via smell only (orthonasal olfaction). Physicochemical analyses show differences among the wines as a function of wine origin, including in elemental composition (e.g., magnesium; sulfur) and in volatile composition. Results pertaining to association of key sensory and physicochemical data will be presented.

Funding support: NZ Ministry for Science & Technology; Lincoln University; University of Burgundy; University of Bordeaux; Pernod Ricard NZ & Pernod Ricard Centre of Research, Paris

Sensory and Chemical Effects of Cross-Flow Filtration on White and Red Wines

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Cross-flow filtration is an increasingly common postfermentation process in the wine industry. Compared to traditional dead end filtration, it is generally faster, requiring only one pass through the filtration unit with a lower chance

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Enology — Sensory/Sensory Impacts Session – CONTINUED

of membrane fouling. Because of the nature of the membranes used in this process, our hypothesis was that cross-flow filtration would not have a significant impact on the sensory or chemical properties of either white or red wines. To investigate this, a California white blend and a California red blend were filtered in three 150 gallon lots using a Bucher Vaslin cross-flow unit with a nominal 0.22 micron polyethersulfone membrane. The unfiltered control was sent directly to the bottling line without filtration. An eight-month descriptive analysis panel was conducted on the wines. Panelists tasted the wines nine times during the eight-month period with replicated tasting at each time point. The Harbertson–Adams assay and UV-Vis with the Skogerson–Boulton algorithm were used to determine phenolic content of the wines. MANOVA indicated the wines changed over time after filtration, a bottle-shock effect that we have observed in previous filtration experiments, but also by treatment. Repeated measures ANOVA was used to account for the significant time effect and the product main effect was found to be significant for two sensory attributes out of 16 total measured in the white wine and six sensory attributes out of 16 total in the red wine. Unfiltered red wines were found to be higher in earthy, grassy, oak, and smoke aromas compared to filtered wines and lower in mixed berry and stonefruit aromas compared to the filtered wines. These differences were most prominent at times longer than one month postfiltration. Unfiltered white wines were found to be higher in hot mouthfeel compared to filtered white wines.

Funding support: American Vineyard Association

Impact of Brown Marmorated Stinkbug on Pinot noir Wine Quality

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The brown marmorated stink bug (BMSB) is an invasive pest that has spread rapidly across the United States (currently in 38 states). This insect has been found in grapes during the harvest period and can contaminate the crop during processing. BMSB creates two problems for wine production. First, the stink bugs have the potential to damage fruit berries. Second, negative impacts on wine flavor can occur when defensive compounds, *trans*-2-decenal and *trans*-2-octenal, are excreted by the stink bugs during processing. The main purpose of the study was to determine if BMSB had an impact on Pinot noir quality when different quantities of bugs were incorporated with the grapes through wine processing. Three treatments were investigated: the control with no bugs in the grapes; treatment 1 (T1) with one bug per four clusters; and treatment 2 (T2) with one bug per two clusters. BMSB excreted defensive compounds during destemming and pressing steps, as a distinct aroma could be perceived. The resulting wines were found to contain higher levels

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Enology — Sensory/Sensory Impacts Session – CONTINUED

of *trans*-2-decenal as well as two additional compounds excreted by the bugs that have yet to be reported, tetradecane and dodecane. *trans*-2-Octenal was undetectable in the wine. The use of difference testing (triangle tests) revealed that T1 and T2 wines were perceived as different from the control ($\alpha = 0.05$). Therefore, BMSB do have effect on wine aroma and flavor if present with the grapes through wine processing, which in turn will impact quality. These results provide information to characterize and minimize this taint to help ensure that the grape and wine industry does not experience the devastation currently found in other food crops due to BMSB.

Funding support: Oregon State University

From Grape to Consumer: Relationships between Grape Maturity, Wine Composition, and Wine Sensory Properties in Cabernet Sauvignon

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Vitis vinifera L. cv. Cabernet Sauvignon wines have been described as presenting a "dichotomy of sensory attributes" and are notably distinguished by the presence of both *green* and *fruity* characters. While some evidence exists that a loss of *green* characters may be associated with wines made from riper grapes, grape ripening is a dynamic process in which multiple biochemical pathways interact to confer changes in berry morphology and the concentration of metabolites for extraction into must and wine. This study undertook a multidisciplinary approach incorporating a suite of chemical and sensory analyses in order to better define the relationship between grape maturity, wine chemistry, wine sensory attributes, and consumer liking response. A series of five triplicate wines was produced from sequentially harvested Cabernet Sauvignon grape parcels, giving a range of alcohol content between 12% and 15%. Wine compositional measures such as dimethyl sulfide, glycerol, isobutylmethoxypyrazine, hexanol, *Z*-3-hexen-1-ol, malic and succinic acids, tannin, ethyl- and acetate esters, higher alcohols, and polysaccharide composition were strongly influenced by grape ripeness. The sensory attributes *dark fruit, hotness*, and *viscosity* increased in wines produced from riper grapes, while *red berry* and *fresh green* decreased. Using partial least squares regression, many sensory attributes were strongly associated with the chemistry data, which indicates scope for understanding the wine chemistry components which are important to wine style and consumer preference. If those components which are important to achieving the desired wine style are better understood in terms of grape maturity, the ability to strategically manage wine style may be improved.

Funding support: GWRDC (Australia)

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Enology — Sensory/Sensory Impacts Session – CONTINUED

Sensory Changes by Partial Dealcoholization Impact of Viticultural and Technological Measures

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In a joint project for the first time both viticultural and technological aspects of partial dealcoholization were investigated in Germany and sensory modifications are reported here. The strongest influence was due to viticultural measures because they were associated with a wide variance in maturity, ranging from 18 to 25 Brix. The treatment of canopy hedging in the summer (18 Brix) was perceived with lower grassy and mushroom characters but stronger mango intensities and less bitterness. Application of antiperspirant oil covering the canopy reduced the must weight from 24 to 22 Brix in comparison to the grape zone defoliation treatment. Bitterness, perception of ethanol as well as body decreased but also significantly peach and mango attributes. Different technologies for partial dealcoholization have been studied in various varieties at different maturity levels. With a light Riesling from the Mosel the early harvest was not convincing due to excessive acid, hard mouthfeel, and thin body. In their olfactory expression neither dealcoholization by membrane technology nor water addition showed systematic differences to the later picked control. However, this control was perceived as bitterer, alcoholic, and with a harder mouthfeel. Ethanol reduction by 2% vol using a hydrophobic membrane changed in Riesling and Sauvignon blanc only the taste properties, while none of the odor attributes changed significantly. This demonstrates the protection for aroma compound during this process. Even stronger taste modifications were observed in a Pinot blanc, which was reduced from 14.6 to 12.6% vol. Sourness increased; bitterness, alcoholic perception, and mouthfeel decreased; and apple and honey flavor were enhanced. Even in the studied Pinot noirs vinified by skin maceration as well as thermovinification few odor changes were observed due to partial dealcoholization. In contrast, reduction of alcohol decreased the intensity of bitterness, astringency, body, and especially alcoholic perception.

Funding support: Projektträger Bundesanstalt für Landwirtschaft und Ernährung

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Viticulture — Environmental Impacts Session

Impacts of Global Warming on Grape Phenology, Vine Growth, and Grape Quality

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This project tested the potential impact of a warmer climate on grape production and quality in a field experiment near Mildura in NW Victoria. Open-top heated chambers were installed in fully established vineyards of Chardonnay, Cabernet Sauvignon, and Shiraz. Temperature in heated chambers was maintained at ~2°C above ambient temperature. Vines growing in heated chambers were compared to vines growing in unheated chambers and vines growing outside the chambers. Vine development, leaf functioning, fruit growth, fruit quality, and yield were closely monitored throughout the season. Wine made from fruit grown in heated and unheated chambers was analyzed and its quality was assessed. Heating accelerated budburst by 3 to 12 days, capfall by 5 to 10 days, and veraison by 5 to 12 days. Heating delayed leaf fall. Modeling that indicated a significant advance in phenology with a relatively small increase in the average daily temperature was therefore confirmed. Leaf canopy function was similar in heated and unheated vines; there was no difference in stomatal conductance or leaf temperature in response to heat. Yields were variable in 2011 due to differential impacts of disease following unseasonal wet weather. No significant impact was seen in 2012. Heating increased the rate of ripening. This was most noticeable for the early ripening Chardonnay in both seasons and for Shiraz and Cabernet Sauvignon in the second season. The influence of temperature on flavonols and tannins remains unclear. The project is currently entering its second phase to test the effect of CO_2 on grapevine development and grape and wine composition and the interaction between CO_2 and temperature in Shiraz grapevines.

Funding support: Department of Primary Industries Victoria

Influence of Cluster Position on Berry Temperature and Composition in Two Merlot Plantings with Different Row Directions

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Effects of cluster position and berry exposure on Merlot composition were determined in neighboring vineyard blocks with rows directed NE-SW and NW-SE. Bilateral-cordon trained vines were thinned prebloom to retain 20 clusters at positions receiving direct solar radiation during an early or later period each day. Fisheye images revealed that in the block with NE-SW rows,

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Viticulture — Environmental Impacts Session – CONTINUED

direct radiation was received in the SE cluster zone between ~0730 and 1230 hr, and in the NW cluster zone between ~1400 and 1700 hr. Surface temperature of exposed berries was higher for SE than NW clusters by up to 7°C between ~0900 and 1700 hr, after which it was higher for NW clusters by up to 3°C until ~2100 hr. Berry mass and soluble solids were similar between SE and NW clusters but exposed berry pH was higher in SW clusters. At both cluster positions, exposed berries had lower soluble solids and titratable acidity and higher pH than did shaded berries within the same clusters. In NW-SE rows, direct radiation was received in the NE cluster zone between ~0730 and 0930 hr and in the SW cluster zone between ~1000 and 1500 hr. Surface temperature of exposed berries was higher for NE than SW clusters by up to 3°C between ~0900 and 1000 hr and was higher for SW than NE clusters by up to 2°C between ~1200 and 1800 hr. NE and SW clusters had similar berry mass, but NE clusters had higher soluble solids and titratable acidity and lower pH. In SW clusters, berries had similar mass but juice pH was higher in exposed than shaded berries, whereas in NE clusters, exposed berries had higher mass but similar composition to shaded berries.

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

Linking Dormancy, Cold Hardiness, and Budbreak: A Model

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Grapevine (*Vitis* spp.) cold hardiness is a dynamic property that varies with species, cultivar, phenology, temperature, day length, and organ of interest. Primary buds follow an overall trend of acclimation following entry into dormancy in fall, maximum but variable hardiness in midwinter, and deacclimation leading up to budbreak in spring. We used long-term (up to 24 years) data sets of lethal bud temperatures, measured using differential thermal analysis, and spring phenology to develop and evaluate a thermal-time model that simulates cold hardiness from the onset of dormancy through budbreak of 23 diverse grapevine genotypes. We established unique model variants for each genotype, using an optimization process with over 1.6 million stepwise iterations to determine genotype-specific parameters, such as initial and maximum hardiness, temperature thresholds, acclimation and deacclimation rates, and chilling and heating requirements. The model uses mean daily temperature as the only input variable to drive daily changes in hardiness. Moreover, because it simulates cold hardiness at budbreak, the model may also be used to predict the time of budbreak. By grouping genotypes into five broad areas of European and North American origin, we found a north/inland-south/coastal gradient in

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Viticulture — Environmental Impacts Session – CONTINUED

terms of initial and maximum cold hardiness, as well as of time of budbreak. Budbreak tended to occur earlier in hardier genotypes. Our model can be coupled with weather forecast services and climate models and may be used by growers as a risk-assessment and decision-aid tool.

Funding sources: Washington State University Agricultural Research Center, Washington Wine Industry Foundation, Chateau Ste. Michelle Distinguished Professorship in Viticulture, WSU AgWeatherNet Program, WSU Viticulture and Enology Program

Impact of Sampling Strategies and Morphological Indicators on Grapevine Cold Hardiness Status

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Ability to survive exposure to dormant season freezing temperatures is a critical economic component of grapevine physiology in cool-cold viticultural regions. Cold hardiness is physiologically complex, involving numerous genetic components. Expression of each component will vary as a result of the interaction between climate and viticulture management. In cool-cold climate viticultural regions the challenge is to culture vines so that the expression of this genetic constitution is maximized. Systematic evaluations of vine tissue hardiness involve specialized equipment and tissue sampling strategies encompassing the large natural variability among tissues and organs in withstanding freezing temperatures. Importantly, up to 15°C variation in tissue cold hardiness exists in every canopy. The key is to assess the frequency of each variation within a given canopy. This work reviews several decades of cold hardiness laboratory work at Michigan State University and suggests a new practical system for viticulturists to assess vineyard treatment effects on cold hardiness. The system associates morphological characteristics of canes and their frequency distribution within canopies with the ability of cane, bud, and perennial wood tissues to survive freeze stresses throughout the dormant season. Results have demonstrated that cane characteristics most predictive of hardiness are periderm color; shoot vigor; persistent lateral status; and number of mature nodes/cane. A cane classification tool (CCT) is suggested for assessing vine cold hardiness based on the frequency of each class within a given canopy. It will be discussed as a practical tool for vineyard managers seeking to assess impact of vineyard practices such as crop level, rootstock choice, training system choice, canopy management, vineyard floor management, and nutritional program on vine hardiness (bud, cane, and perennial wood) as assessed by a frequency distribution of the desired (and undesired) cane characteristics.

Funding support: Michigan Grape and Wine Industry Council, Project GREEEN at Michigan State University, Viticulture Consortium–East

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Viticulture — Environmental Impacts Session – CONTINUED

Increased Freezing Tolerance of Grapevines Linked to Abscisic Acid-Induced Bud Desiccation and Sugar Accumulation

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Grapes are temperate crops and are most frequently damaged by freezing temperatures. The severe economic losses that result from the freezing injury continue to be a major problem to the grape and wine industries in cold regions. The goal of this research was to develop a novel method to improve the freezing tolerance (FT) of grapevine using abscisic acid (ABA) and further our understanding on possible physiological and biochemical mechanisms. Recent work indicated that exogenous ABA application can advance cold acclimation and dormancy of grapevines, which resulted in increased FT in cold sensitive cultivars. In this study, it was hypothesized that FT is linked to ABA-induced tissue desiccation and soluble sugar accumulation. The specific objectives were to evaluate changes in FT, water content, and soluble sugar concentration in grapevine buds under field and greenhouse conditions and determine the associations among those responses. ABA was applied on potted and field-grown Cabernet franc and Chambourcin grapevines. Thermal analysis and gas chromatography were used to measure FT and soluble sugar concentration, respectively. ABA treatment increased the FT, decreased water content, and increased total soluble sugar concentration in buds. Specific sugars showed different relationships with FT during the acclimation stage and midwinter; thus, alternate roles of these sugars are suggested. It is concluded that ABA has the capacity to enhance dormancy and increase FT and can be utilized as a prophylactic tool to protect sensitive grape cultivars from cold damage.

Funding support: HCS-OARDC/OSU, Ohio Grape Industries Committee, Valent BioSciences Co.

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Enology — Flavor: Impact of Yeast and Bacteria Session

Identification of a New Genetic Mechanism Providing Simultaneous Control of the Production of SO_2 and H_2S by Wine Yeasts

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Sulfites are widely used in enology. However, trends tend to reduce their use and there is a strong requirement to control their final amount. Apart from voluntary use, sulfites can also be produced by wine yeasts in significant amounts during grape must fermentation, which can lead to a delay in the onset of malolactic fermentation and to a lack of control of its final concentration. Furthermore, sulfites are precursors of the synthesis of sulfide, a highly undesirable by-product. Wine yeast can highly differ in their capacity to produce these sulfur compounds; however, the molecular bases of such differences are not known. We have set up a genetic study to identify the molecular bases of these properties. Using a quantitative genetic approach, we could map two loci impacting SO_2 and H_2S production. Dissecting the QTLs led to the identification of two alleles of the genes *MET2* and *SKP2* responsible for the differences in sulfur compound production between two wine yeast strains. A functional validation demonstrated their implication and highlighted the strength and the extent of their control over the phenotypes. Indeed, the *MET2* gene governs the availability of *O*-acetylhomoserine, precursor of the incorporation of H_2S into carbon chains, whereas *SKP2* is responsible for the control of the stability of Met14p, a key enzyme of the sulfate assimilation pathway. Their combination triggers a simultaneous control of both branches of the sulfur metabolism, and we could hypothesize that their mechanism of action is strong enough to exert a complete control in any yeast strain presenting excessive production of SO_2 and/or H_2S. The transfer of those alleles by breeding will offer great opportunities for further strain improvement, and a new wine yeast strain has already been constructed using molecular marker assisted-selection.

Funding support: INRA Montpellier Supagro

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Enology — Flavor: Impact of Yeast and Bacteria Session– CONTINUED

Impact of *Oenococcus oeni* on Wine Hydroxycinnamic Acid Content and Production of Volatile Phenols by *Brettanomyces*

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Degradation of hydroxycinnamic acids and their tartaric esters by *Oenococcus oeni* and the subsequent impact on production of volatile phenols by *Brettanomyces bruxellensis* was investigated. Pinot noir wine was produced and sterile filtered with no addition of SO_2. Wines were inoculated with one of three commercial *O. oeni* strains and an uninoculated control was also prepared. Bacterial growth and malic acid was monitored while hydroxycinnamic acid content was assessed by HPLC-DAD. At the completion of malolactic fermentation (MLF) no change was noted in the concentration of caffeic, caftaric, *p*-coumaric, or coutaric acid in wines inoculated with *O. oeni* strain #2 and strain #3. However, in wines inoculated with *O. oeni* strain #1, there was a decrease in the concentration of coutaric acid from 9.9 to 3.1 mg/L with a corresponding increase in *p*-coumaric acid from 1.4 to 6.3 mg/L. All wines were then inoculated with *B. bruxellensis* UCD 2049 at ~1 x 10^3 cfu/mL. Growth was monitored by plating with populations of *B. bruxellensis* reaching ~1 x 10^6 cfu/mL in all treatments after 35 days with wines being assessed for volatile phenols by GC-MS/MS after 42 days. *B. bruxellensis* metabolized *p*-coumaric acid but was unable to metabolize coutaric acid. Because of this *B. bruxellensis* produced significantly higher 4-ethylphenol (4-EP) in wines in which *O. oeni* strain #1 had grown due to the higher initial concentration of *p*-coumaric acid. For example, while the control wine contained 263.3 mg/L 4-EP, wine that underwent MLF with *O. oeni* strain #1 contained 1579.5 mg/L 4-EP. This wine also contained more than three times the concentration of 4-ethylguaiacol (4-EG). Wines that underwent MLF with *O. oeni* strain #2 and #3 contained similar 4-EP and 4-EG concentrations to the control.

Funding support: NW Center for Small Fruits Research

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Enology — Flavor: Impact of Yeast and Bacteria Session– CONTINUED

Production and Accumulation of Diacetyl in White Wine by Yeast (*Saccharomyces cerevisiae*) and Bacteria (*Oenococcus oeni*)

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Diacetyl is primarily formed during malolactic fermentation (MLF) due to citrate catabolism by lactic acid bacteria. Bacterial citrate lyase usually starts the synthesis. However, former experiments have shown that even in vinifications with citrate lyase negative strains (CL_{neg}) significant amounts of diacetyl can be produced. The present study was designed to evaluate different pathways of diacetyl formation in yeast and bacteria and their role in its accumulation. Small-scale fermentations were performed in two vintages with five commercial bacterial starter cultures. The strains were selected according to their ability to form diacetyl and were divided into strong producers, medium producers, and CL_{neg} bacteria. Gene expression levels of five critical metabolic steps in bacteria were monitored relative to lactate dehydrogenase as house-keeping gene. Diacetyl was analyzed by GCMS and citrate was monitored enzymatically. Fermentation and MLF parameters were analyzed by FT-MIR spectroscopy. The results show a significant influence of cell concentration on overall metabolic performance. In some commercial products it seems to be difficult to reach the critical cell mass when prepared according to manufacturer's instructions. However, there are always two peak concentrations of diacetyl during the vinification process. The first peak occurs after two or three days and is produced by fermenting yeast in connection with *Oenococcus oeni*, which shows a high overexpression of diacetyl related genes but no accumulation because of the high yeast activity at that point. The second peak corresponds to citrate degradation after MLF. In both cases the intermediate product pyruvate seems to play the central role since it can be produced and used by both yeast and bacteria. High pyruvate levels might trigger the secondary pathway of diacetyl synthesis in both groups of microorganisms. Consequently, the formation cannot be prevented completely. The challenge is to regulate its accumulation, for which some methods could be identified.

Funding support: Rhineland-Palatinate Ministry for Environment, Agriculture, Nutrition, Viticulture, and Forestry

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Wednesday National Conference
Oral Presentation Abstracts (Research Reports)

Enology — Flavor: Impact of Yeast and Bacteria Session– CONTINUED

Brettanomyces bruxellensis Aroma Impact Wheel

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Brettanomyces yeast are a major spoilage issue in wines throughout the world. However, many premium wines contain some *Brettanomyces* character that is highly prized for the complexity it adds to wines. We have done extensive chemical and olfactory analysis of wines by solid-phase microextraction with mass spectrometry and olfactory detection to determine the types of aromas that are associated with different *Brettanomyces* strains in a synthetic medium supplemented with cinnamic acids and aromatic amino acids as substrates. The descriptors that were most often used when describing the aromas produced have been compiled into an aroma wheel. Although some of these characters are clearly negative, many can add positive aromas to the wine. This wheel was initially developed for model wine but the major categories were confirmed in actual wine infected with different strains of *Brettanomyces*. These or similar terms are often used in wine reviews and marketing descriptions to describe wine attributes. Thirty-three commercial wines were identified by searching for some of the terms of the aroma wheel in databases of wine reviews on online sites. Microbiological analysis found that more than 60% had viable lactic acid bacterial contamination and 30% had culturable *Brettanomyces*. Some of the wines not yielding viable *Brettanomyces* had 4-ethylphenol levels indicative of *Brettanomyces* activity and were likely filtered. Wild lactic acid bacteria and *Brettanomyces* are often found together in wines and can make the same spectrum of compounds from amino acid precursors. The fact that most of these wines had viable bacteria and/or yeast indicates that many of these descriptors may be more universal indicators of wild microbial populations found in these wines rather than strictly associated with *Brettanomyces*.

Funding support: American Vineyard Foundation, California Competitive Grant Program for Viticulture and Enology, UC Davis Department of Viticulture and Enology Wine Yeast and Bacteria Collection

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Viticulture — General Session

The Consequences of Variable Bunch Architecture for *Vitis vinifera* L. Sauvignon blanc Grown in New Zealand

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Bunch architecture is influenced through environmental and genetic factors during the course of two consecutive growing seasons. A highly variable component of bunch architecture is the outer arm. The presence of an outer arm causes an increase in yield, but has the potential to cause significant differences in fruit composition when compared to the main rachis at harvest. To investigate the variability of bunch architecture we studied cane-pruned (vertical shoot-positioned) Sauvignon blanc vines over two growing seasons. Cane-pruned vines were chosen as the node positions along a cane exhibit an inherent temporal variability in inflorescence initiation and development. Our results indicate that temperature during inflorescence initiation has a major influence on subsequent bunch architecture. As temperatures during initiation increase, bunch number per shoot and the occurrence of an outer arm in the subsequent season also increase, while bunches on the developing shoot are more basally located. Inflorescence position on the shoot also determines flowering progression. The main rachis of basal inflorescences flower first, followed by the apical inflorescence. The outer arm component flowers after the inner arm. The delay in flowering between bunch positions along a shoot and bunch components is mirrored in their soluble solids concentration at harvest. The timing of flowering determines the variation in soluble solids concentration at harvest both within and between bunches and cannot be overcome. The delay in flowering between the inner and outer arm component is reflected in the relative difference in berry number between the two components. Bunches with similar berry numbers between the two components have more synchronous flowering and uniform soluble solids at harvest. Our study gives insight into the consequences of variation in bunch architecture and provides a basis for future work regarding the influence of temperature on outer-arm development.

Funding support: Designer Grapevines Research Programme, funded by the New Zealand 453 Ministry of Science and Innovation (contract CO6X0707) and Plant & Food Research

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Viticulture — General Session – CONTINUED

Vegetative and Reproductive Parameters Evaluation of "Alternative" Plant Material

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Dead vines are often a problem in many vineyards and are due to physiological or pathological causes whereby the vines need to be replaced. The replacement operation is a real cost for the direct costs of the plant material, for its planting and for their different management during the current year or in the two years after the replacement, determining also vineyard variability. When replacement is performed in the first years after vineyard establishment it is quite easy, whereas many issues occur when replacement is later, particularly issues considering root competition. To reduce replacement problems, the use of "alternative plant material" has been evaluated. The alternative vines were one year old with a shoot length ~80 to 100 cm and a shoot diameter no less than 7.50 mm. Such plant material has higher producing costs than normal vines, but it has a shorter and easier training period. The use of alternative vines also can be a useful tool in varietal changing (in addition to or in replacement of the graft techniques) to obtain a moderate yield since the plantation year. In this case the cost-effectiveness should be evaluated carefully. The research conducted during the 2011 and 2012 growing seasons studied the cultivation techniques adopted in the nursery to obtaining suitable alternative plant with different graft combinations. Vegetative and productive parameters of alternative vines during the first year of planting were also evaluated. Results suggest different vineyard management compare to the traditional management. Particularly, in the nursery, vines seemed to be more vigorous when there is a greater distance in the row and foliar fertilization is applied. During the first year of planting, good vegetative and reproductive performances were shown. When bunches were left on high-vigor vines, they ripened properly without negative effects on vegetation development.

Funding support: University of Palermo

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Viticulture — General Session – CONTINUED

Mechanical Canopy Management Reduces Labor Costs and Maintains Fruit Composition in Cabernet Sauvignon Grape Production

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Three canopy management methods—hand pruning (HP), mechanical pre-pruning with hand shoot thinning (MP+HT), and mechanical box-pruning with mechanical shoot thinning—were applied with the objective of achieving similar and commercially marketable Cabernet Sauvignon grape (*Vitis vinifera*) yields while maintaining vine balance and comparing labor operations costs. Canopy management system labor operation cost estimates indicated a 62% and 80% labor savings with the MP+HT and MP+MT treatments, respectively, when compared to HP. The total shoot density of the vines was not affected by the treatments applied. However, the contribution of count shoots increased with the concomitant addition of mechanization to canopy management. All treatments achieved similar canopy architecture and microclimate. The treatments did not affect photosynthetically active radiation intercepted in the fruiting zone of canopy at veraison. All treatments had similar yield, total soluble solids, juice pH, and titratable acidity at harvest. Berry skin total phenolics, anthocyanins, and tannins when measured at harvest were also similar among the treatments applied. All treatments tested were within acceptable Ravaz index limits of 5 to 10 kg/kg. However, only MP+MT treatment reached a near optimum leaf area to fruit ratio of 1.2 $m^2 \cdot kg^{-1}$ and pruning weight of 1.0 $kg \cdot m^{-1}$ for warm climate viticulture. The results of this study provide commercially acceptable mechanical canopy management options that may provide labor cost savings for winegrape growers in the San Joaquin Valley of California.

Funding support: ARI Bronco Wine Company Research Trust Oxbo International

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Viticulture — General Session – CONTINUED

Crop Load Mapping in Concord with On-the-Go Proximal Sensors

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Crop load is a source:sink concept in viticulture. It hypothesizes that optimum production conditions can be defined by the ratio between the source (vine size/leaf area) and sink (yield) of carbohydrate in the vine, rather than the absolute values of the source/sink. Recent work has shown that this concept is particularly applicable in winegrape production and the crop load metric can be used to help in making correct spatial management decisions. However, to map and spatially manage crop load, spatial information on both the source (vine size) and sink (yield) size is required. NGWI has been supporting research to adapt existing agricultural sensors and to develop novel viticultural sensors for the purpose of within- and postseason crop load mapping. Here we address the former issue of how to adapt existing sensors. Over the past few years, sensor evaluation and protocol development has been undertaken in Concord vineyards in western New York to determine the suitability of existing, commercially available proximal sensors for crop load mapping. The developed approach uses a strategically positioned, proximal, optical canopy sensor, linked to a GPS receiver, to map canopy vigor at key phenological stages. The canopy vigor information is then used to develop a stratified sampling scheme to measure pruning weights postharvest to calibrate the sensor response to vine size. Sink size (yield) is measured by a harvester-mounted load-cell yield sensor, again linked to a GPS receiver. The results show that there is great variability in vine size and yield within Concord vineyard blocks, which is consistent with results from similar work in other viticulture systems, as well as a great variability in crop load. Error sources and the accuracy and precision of measurements of vine size, yield and crop load will be discussed in the context of the information needed for crop load management.

Funding support: National Grape and Wine Initiative

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Enology — Wine Stability and Oxidation Session

Temperature Effects on Membrane Lipid Composition and Fermentation Outcome in Wine Yeast

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A wine fermentation is an ever-evolving environment that produces numerous and diverse stresses on yeast that require them to adapt to changes in osmolarity, increasing levels of toxic byproducts of metabolism, and thermal fluctuations to grow, reproduce, and survive. Extremes of fermentation temperature often result in fermentation arrest under growth conditions that would otherwise result in complete sugar utilization at "normal" temperatures and nutrient levels. A contributing factor to fermentation arrest is the inability of the yeast strain to tolerate or adapt to increasing ethanol concentrations, and exposure to temperature extremes can exacerbate ethanol's effect on membrane bilayers. While there is significant evidence the lipid composition of yeast contributes to ethanol tolerance, it is less clear how the membrane composition adapts to different fermentation temperatures. Small-scale fermentations were carried out with three industrial yeast strains, including wine yeast strains that exhibited markedly different sugar utilization and ethanol tolerance characteristics in a defined synthetic grape juice. The fermentations were performed at three different temperatures commonly encountered during wine fermentations. Lipids were extracted from yeast at numerous fermentation time points using a modified Bligh–Dyer method developed by our group. Phospholipids were quantitatively analyzed via a normal-phase liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) method developed by our group. Flow injection analysis by atmospheric pressure chemical ionization–mass spectrometry (APCI-MS) was employed to quantitatively determine sterol concentration in yeast lipid extracts. Lipid structure and identity were confirmed using tandem mass spectrometry and fragmentation databases developed by our group. Differences in lipid membrane components based on fermentation temperature will be discussed in the context of the relationship between lipid composition and successful completion of fermentation. The data will be used to modify the ethanol tolerance of wine yeast strains with other favorable flavor or quality attributes.

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Thursday National Conference
Oral Presentation Abstracts (Research Reports)

Enology — Wine Stability and Oxidation Session – CONTINUED

Effects of Transition Metals on the Evolution of Volatile Sulfur Compounds in Wine during Bottle Storage

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Reduced aromas caused by volatile sulfur compounds (VSCs) can impact negatively on the aroma of wine. Boiled or rotten egg, sewage, and rubber are descriptors associated with these VSCs. The pool of potential precursors to VSCs in wine is extensive, and many sulfur-containing molecules are present in mg/L concentrations, while VSCs start to become problematic at µg/L concentrations. For example, methionine is present in wine in concentrations ranging from 1 to 45 mg/L. This makes it important to understand not only the formation of VSCs from precursors but also the mechanism, or switches, driving the release of VSCs from various precursor molecules. Investigating the role of metal ions as catalysts, as well as the synergistic effects of the metals during their catalytic action, in the formation of VSCs are crucial to gain a full understanding of the chemical processes governing the formation of postbottling "reductive" aromas. In this study we have investigated the formation of VSCs, specifically methanethiol (MeSH) from methionine during wine maturation, as catalyzed by five transition metals (Al, Cu, Fe, Mn, and Zn) normally present in wine and that are known for their catalytic ability. Wines were stored under anaerobic conditions and analyzed at five time points over a 12-month period. Dissolved oxygen was monitored during the experiment to study the effect this had on the wine chemistry. The evolution of hydrogen sulfide (H_2S), MeSH, and dimethyl sulfide (DMS) were directly influenced by different metals, and in some instances a combination of metals were responsible for the greatest increase in VSCs concentration. Copper showed a strong correlation with MeSH.

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Controlling Wine Oxidation by Understanding Antioxidant Availability and Reactivity

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Wine oxidation creates reactive electrophiles such as quinones and other carbonyls, aldehydes, and ketones. These will react with whatever nucleophiles are present in the wine. Any of these—SO_2, glutathione (GSH), ascorbic

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acid, or phenolics—can act as natural protection from oxidation. The ability of these preservatives, and important aromatic thiols, to react with quinones was analyzed by competitive reactions rate with 4-methyl-1,2-benzoquinone (Q4MeC), a model quinone. Based on the results, SO_2, ascorbic acid, and glutathione react quickly as sacrificial nucleophiles, potentially providing protection against loss of 3-mercaptohexanol, a key aromatic thiol in many wines. The reactivity of SO_2, ascorbic acid, and GSH were very similar, and no antioxidant synergic effect was observed in mixtures. ^{1}H, ^{13}C, and 2D NMR analyses were used to identify the reaction products between Q4MeC and the wine nucleophiles. The availability of sulfur dioxide to act as a preservative appears to decrease with the presence of carbonyl compounds. Some are well known to form nonprotective, bound SO_2, but the effects of others are ambiguous. To fully understand and predict the protective potential of SO_2, their role needs further definition under enological conditions.

Funding support: American Vineyard Foundation

Sulfur Dioxide–Oxygen Consumption Ratio Reveals Differences in Post-Bottling Oxygen Development in Chardonnay Wines

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The availability of oxygen to a wine after bottling can have both beneficial and detrimental effects over time, and the effects can be altered by a wine's history. A Chardonnay wine was treated four ways: aged in stainless steel with and without lees and aged in oak barrels with and without lees. After 6-month aging, the wines were bottled and subjected to four levels of oxygen availability by varying the closure. The wines were monitored for dissolved and headspace oxygen, total consumed oxygen, SO_2, aldehydes, esters, and many standard endpoints. After bottling with substantial oxygen uptake, oxygen consumption was slow with 0.5 mg/L dissolved oxygen persisting in some bottles after 6 months. Sulfur dioxide decreased and absorbance increased in response to higher accumulated oxygen exposure, expressed as total consumed oxygen (TCO). A comparison of the oxygen versus sulfur dioxide consumed by the wine provided new insight into the development of oxidation products. Wines aged on yeast lees consumed more SO_2 for each equivalent of oxygen consumed. As this wine also had lower levels of weak SO_2 binding agents, we hypothesize that these substances interfere in protection by that preservative. The ratio of SO_2 versus O_2 consumed may help identify wines that will develop oxidation products.

Funding support: Nomacorc, LLC

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Thursday National Conference
Oral Presentation Abstracts (Research Reports)

Viticulture — Cultural Practices Session

Multi-Season Effects of Rootstock and Intrarow Cover Crops on Vine Growth and Fruit Composition of Cabernet Sauvignon

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The humid growing seasons of the mid-Atlantic United States often foster excessive vine vegetative growth which increases disease pressure and can compromise wine quality potential. Although the consequences of increased vigor can be countered with canopy management practices, we sought proactive means to avoid those consequences. This study investigated the effectiveness with which vine growth could be regulated and fruit quality improved using three different rootstocks (Riparia Gloire, 420-A, and 101-14) and two different intrarow floor management schemes (herbicide strip vs. perennial grass cover crop, creeping red fescue, *Festuca rubra*). We hypothesized that the intrarow cover cropping would have a greater impact on vine size suppression than would rootstock and that the aggressive use of cover crops would improve fruit composition and wine quality potential. Vines grown with intrarow cover crops or on *riparia* rootstock consistently decreased dormant cane pruning weights. Intrarow cover crops affected enhanced point quadrat analysis (EPQA) metrics in a way that related to less shaded fruit zones. Treatment effect on crop yield and berry weight tended to diminish over time, but intrarow cover crop and 101-14 rootstock consistently reduced cluster weights. Primary fruit chemistry was inconsistently affected by intrarow cover crop and rootstock. Intrarow cover crop increased while 420-A rootstock decreased estimated berry skin phenolics, anthocyanins, and color density in the first two of three years. Vintage tended to have a greater impact on most responses than did groundcover or rootstock. Averaging across vintage, intrarow cover crops limited vegetative growth and tended to increase berry soluble solids and estimated skin phenolics, while 101-14 rootstock tended to promote vegetative over reproductive growth, but improved estimated berry skin color density and anthocyanins. Under our conditions, intrarow cover crops appeared to offer a more consistent suppression of vine vegetative growth than did rootstock choice.

Funding support: Virginia Wine Board

The Impact of Undervine Vegetation Management on Vine Performance and Fruit Development

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Herbicide sprays are commonly used in vineyards to keep the undervine region devoid of competing vegetation. However, many growers are looking to reduce their reliance on synthetic chemicals, and thus are seeking nonchemical means

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to control growth under the vines. An investigation into how changing undervine vegetation management alters the performance of the vines is therefore warranted. Three treatments were imposed in Merot and Syrah vineyards in Hawke's Bay and Sauvignon blanc and Pinot noir vineyards in Marlborough, New Zealand: continued herbicide use, undervine mowing, and undervine cultivation. Vine vigor, as measured by leaf area and percent canopy gaps, was generally reduced by the mowing treatment, but unaffected, or even slightly increased, by cultivation. Vine water status, as measured by stem water potential, showed a trend for the undervine mowed treatments to be slightly more water stressed than both the herbicide and cultivated treatments, but these differences were not always statistically significant. Veraison was significantly slower in the mowing trial, whereas there was no difference between the other two treatments. These data show that undervine mowing reduced vigor of the vines and slightly delayed veraison, whereas undervine cultivation had less of a negative effect on vine growth. Therefore, the means of nonchemical undervine management needs to be carefully considered with respect to its subsequent effect on vine performance and fruit development.

Funding support: New Zealand Winegrowers, New Zealand Ministry of Science and Innovation

Factors Affecting the Levels of 3-Isobutyl-2-Methoxypyrazine and C_6 Compounds in *Vitis vinifera* L. Merlot

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A study concerning seasonal and regional variability showed that fruit IBMP and C_6 compounds at harvest are not necessarily higher in cooler climates compared to warmer regions. Significantly higher levels of green aromas were observed in the warmest and driest of the seasons (i.e., 2007) rather than the coolest and wettest (i.e., 2010). Principal component analysis identified the temperature during spring (GDD) as a key factor explaining these results, likely due to the direct effects of spring temperature on vine growth, and indirect effects of fruit shading. A second study focused on irrigation and fertilization practices and showed again the significant effect of vine vigor on IBMP levels in fruit and wine. In contrast, C_6 compounds were not responsive to these treatments. Furthermore, a third study focused on winter rainfall, or the lack of; a factor not previously considered in relation to fruit and wine green aromas. Exclusion of winter rainfall had a very significant impact on IBMP fruit and wine levels, but again C_6 compounds were found to be nonresponsive to the experimental treatments. A severe imbalance between vegetative and reproductive growth was observed for vines under rainfall exclusion conditions. Significant effects were also recorded for vine yield components.

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The studies conducted highlight the importance of conditions promoting vine growth as the main drivers of IBMP fruit and wine levels, mainly due to their negative effects on fruit exposure to light. Therefore, to achieve low IBMP levels at harvest, vineyard management practices that do not promote excessive growth during the spring are required. Otherwise, delayed harvest and decreased yield (due to berry dehydration) would be expected. This work has demonstrated that C_6 compounds do not respond to vineyard management practices.

Funding support: E & J Gallo Winery

Effect of Plastic Cover Materials on Canopy Microclimate and Fruit Quality of Table Grapes

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California table-grape growers cover the canopies of late-season varieties with plastic films to protect the fruit from rain. Films of different color and transparency are commonly used, but whether those differences may affect canopy microenvironment and fruit quality has not been determined. In late September, Redglobe (in 2011) and Autumn King (in 2012) table grape vines were covered with green or white plastic films, or left uncovered, and canopy microclimate, rot incidence, and fruit yield and quality at harvest, and after postharvest storage, were evaluated. The green film was more transparent and less reflective than the white. The films had little effect on fruit zone temperatures, but the daily maximum temperature in the top center of the canopy of vines covered with green film was consistently 5°C higher than that of vines subjected to other treatments. Treatment effects on relative humidity depended on location within the canopy and time of day, but both films consistently reduced evaporative potential under the covers, though not in the fruit zones. Treatment effects on condensation beneath the films were inconsistent, but south-facing surfaces generally had less condensation than vertical or north-facing surfaces. Approximately 1 inch of rain fell on 4 October 2011, but no rain occurred during the experimental period in 2012. Green films slightly delayed fruit maturation in 2011, but not in 2012. Films did not affect the number of boxes harvested, or postharvest fruit quality in 2011, but fruit from covered vines had less postharvest rot in 2012 than fruit from noncovered vines, even though significant rain occurred in 2011 but not 2012. In summary, the films had some consistently different effects on canopy microclimate, but additional data are needed to determine whether effects on fruit quality and rot consistently differ among films and varieties.

Funding support: California Table Grape Commission

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Enology — Tannins Session (Part I)

Which Tannins in Grapes Are Important for Wine?

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The usefulness of measuring tannin in grapes and wine and its uptake by the wine industry is limited by a lack of understanding of how grape tannin measures relate to tannin in wine. The concentration of tannin in grapes measured at harvest is much different than the concentration of tannin in the final wine, as the amount of tannin extracted is influenced by many factors including the interactions of tannin with cell walls and the fermentation conditions during winemaking such as temperature and duration of skin contact. Historically, tannin measures have focused on determining total tannin, but only a portion of the total grape tannin is measured in the wine. To investigate the discrepancy between grape and wine total tannin measures, this study examined the distribution of tannin in the skin and seeds of Shiraz and Cabernet Sauvignon winegrapes and wine made from those grapes to determine the type and amount of grape tannin extracted into wine. The influence of cell walls, anthocyanin and solvent on tannin extraction from grape skin was also investigated. While skin tannin distribution ranged between a polymer length of 3 subunits to greater than 60 subunits, the wine tannin distribution was much less, ranging between a polymer length of 3 and less than 20 subunits. It is likely grape tannin greater than 20 subunits is not extracted into wine determined by both its interactions with cell walls and wine conditions. While we can measure a large amount of total grape tannin, the distribution and concentration of grape tannin below a polymer length of 20 is important for determining the tannin that is extracted into wine. If tannin below a polymer length of 20 is not extracted into wine, then further research to determine which tannins are important in wine should focus on the tannins that are actually extracted into wine rather than the total available pool.

Funding support: Department of Primary Industries Victoria and the Grape and Wine Research and Development Coorporation

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Enology — Tannins Session (Part I) – CONTINUED

Interactive Effect of Regulated Deficit Irrigation and Skin Contact Time in Washington State Cabernet Sauvignon Wines

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A Cabernet Sauvignon vineyard was subjected to four regulated deficit irrigation (RDI) regimes: (1) 100% replenishment of evapotranspiration (100% ET_0); (2) 70% ET_0; (3) 25% of ET_0 until veraison, followed by a 100% replenishment of ET_0 until harvest; and (4) 25% of ET_0. To study the interaction between RDI alternatives and maceration length, two vineyard replicates were designated as controls (C, 10-day skin contact) and two as extended maceration (EM, 30-day skin contact). The size distribution, concentration, and composition of wine proanthocyanidins (PA) and flava-3-ols were characterized. Additionally, a trained panel generated a descriptive analysis of the wines. The maceration length imparted a greater effect on most chemical and sensory parameters and determined a balanced extraction from skin and seeds in control wines and an enhanced extraction from seeds (~73%) in EM wines. The size distribution of the wine PAs revealed two major peaks as a function of concentration at mDP 2 (22 to 27% of total PA mass) and 6 to 7 (12 to 17% of total PA mass) and was found to follow a Rayleigh-type, non-normal distribution. The RDI 100% ET_0 treatment resulted in wines with lower ratings of color, fruity aromas, and mouthfeel attributes. The wines of the 70% ET_0 and 25/100% ET_0 treatments were defined by red and black fruit aroma and the 25% ET_0 treatment generated wines with higher purple component and saturation. Control wines were more purple, saturated, and with enhanced black and red fruit aromas. EM wines were less saturated, with higher ratings for brown component, oxidized character, astringency, and bitterness. Bitterness ratings increased along with the flavan-3-ol content of the wines. Astringency ratings increased along with the total PA content of the wines and, to a lesser extent, with the content of monomeric flavan-3-ols, the latter implying that a relatively high concentrations (>288 mg/L) monomeric flavan-3-ols may elicit astringency.

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Enology — Tannins Session (Part I) – CONTINUED

Tannin Extractability among Hybrid and *Vitis vinifera* Grapes: Cultivar Variability and Differential Binding to Cell Walls

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Anecdotally, wines produced from interspecific hybrid grapes have low astringency, and increasing tannin content of these wines is an interesting target for grape breeders. However, it is not known if these purported low tannin concentrations are due to low tannin content of interspecific hybrid grape berries or to poor extraction during winemaking. To investigate this, six hybrid grape varieties (Baco noir, Leon Millot, Marechal Foch, DeChaunac, Corot noir, and Noiret) and six *Vitis vinifera* varieties (Pinot noir, Lemberger, Sangiovese, Cabernet Sauvignon, Cabernet franc, and Merlot) were harvested separately across two locations in the Finger Lakes region of New York and fermented under similar conditions. Tannins in skins, seeds, and resulting wines were measured by a protein precipitation assay. The concentration of tannin in wines produced from interspecific hybrids was significantly lower than those produced from *vinifera* grapes (91 versus 248 mg/L CE, $p < 0.05$). In agreement with previous literature, it was observed that both total grape tannin by weight ($r^2 = 0.18$) and grape skin tannin were poorly correlated with wine tannin, suggesting that differences may be due to tannin extractability. Cell wall fractions from the skins and flesh of the 12 grape varieties were investigated for tannin binding ability via incubation with commercial tannin preparation in model wine. Cell wall material from the skin of hybrid grapes bound two- to fourfold more tannin on average than corresponding material from *vinifera* grapes.

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Enology — Tannins Session (Part I) – CONTINUED

The Effect of Fruit Maturity, Ethanol Concentration, and Maceration Length in Washington State Merlot Wines

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This work evaluated the effect of fruit maturity on wine phenolic and sensory composition, under different ethanol (EtOH) concentrations and regular (10 days of skin contact) or prolonged maceration (EM, 30 days of skin contact). Merlot grapes from the same vineyard were harvested 33 and 34 days apart in 2011 and 2012, respectively. At each harvest, half of the must was adjusted to emulate the other harvest's soluble solids content by chaptalization and/or by juice removal followed by water (de-chlorinated and acidified) addition. In general, the 2011 season was higher in anthocyanins whereas the 2012 season was higher in seed and skin tannins. As a result, wine anthocyanin extraction was greater in 2011 and tannin extraction was greater in 2012, but specific effects occurred as a function of the maceration technique. For example, consistently lower levels of monomeric anthocyanins and higher percentages of overall and seed-derived tannin were found in EM wines. Moreover, an overall trend of increased tannin extraction from early to late harvest wines was driven by increased extractability from seeds. Extraction of seed tannins was unaffected by the EtOH content by chaptalization or water-back up to 2.7% (v/v). A sensory descriptive analysis differentiated the wines as a function of the maturity level and as a function of the EtOH adjustment and maceration. Wines made from ripe fruit were sweeter with a viscous mouthfeel. Conversely, wines made from unripe fruit were lighter in color, with yellow and red color components, a sour mouthfeel, and vegetal notes. In general, chaptalization increased perceived astringency, whereas water-back decreased bitterness and hot mouthfeel but increased earthy notes in the finished wines. Relative to maturity and EtOH adjustment, EM had less impact but shifted the profile toward more astringency, lighter and yellower color components, and pronounced cooked vegetal aromas.

Funding support: Washington Wine Grape Funds, Wine Advisory Committee, Washington Wine Commission, WSU Agricultural Research Center, Fulbright Commission and the Walter Clore Scholarship (for LFC), and Ste. Michelle Wine Estates

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Viticulture — Water Relations Session

Soil Salinity Survey of Paso Robles Vineyards from 2006 through 2012

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Soil salinity conditions are often an important factor affecting crop growth in arid regions that utilize supplemental irrigation, particularly when the irrigation is with groundwater of variable quality. High salinity levels can significantly reduce vegetative growth and fruit production or may lead to phytotoxicity with reductions of overall yield and quality. If natural rainfall leaching or irrigation leaching fractions are insufficient, or if irrigation water quality is degrading over time, then soil salinity levels can be expected to gradually increase. The purpose of this project was to assess the current soil salinity conditions as well as any changes of these conditions over time in mature vineyards east of Paso Robles, which utilize the local groundwater basin for irrigation. Measurements were first conducted in the late summer of 2006 and were repeated at the same locations in 2007, 2009, and 2012. Composite 30 cm deep soil cores were sampled in the vine row at 100 vineyards; each subsequent sampling was repeated at the same location in each vineyard. This six-year period included both dry and wet winters, with the average rainfall over this period being slightly greater than the 60-year average, measured at the city of Paso Robles. The overall soil salinity level as indicated by the soil electrical conductivity increased steadily throughout the sampling period; by 2012, 57% of the samples exceeded 2 dS/m, and 29% exceeded 4 dS/m. Sodium, calcium, and magnesium levels increased steadily throughout the sample period. Boron was tested in 2009 and 2012; levels exceeded the 0.75 ppm toxicity threshold in 20 and 23% of the samples for the two years accordingly. These results indicate the need for growers in the region to conduct their own assessments of soil salinity and irrigation water chemistry and to adjust cultural practices as necessary for their particular conditions.

Funding support: American Vineyard Foundation

Grape Berry Water Relations: Why Berry Transpiration and Xylem Backflow Matter

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Irrigation close to harvest is thought to be detrimental to grape quality due to a "dilution" effect. Surprisingly, little scientific evidence is available to support this belief. Therefore, we carried out a series of experiments to test it. Using a dye to track water flow via the xylem, we demonstrated that the predominant flow direction changed from inflow into berries to backflow from berries at

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Viticulture — Water Relations Session – CONTINUED

veraison. We also found a distal-to-proximal gradient in soluble solids inside the ripening berries. This, together with our observation that pressurization of the xylem restored dye penetration into the berries, suggests that the flow reversal is caused by a hydraulic pressure gradient within the berries. Owing to the increasing sugar concentration in ripening berries, excess phloem-derived water needs to be disposed of, either via the xylem (backflow) or the skin (berry transpiration). The rate of sugar accumulation in ripening berries decreased, yet cracking incidence increased when xylem backflow and/or berry transpiration were restricted. Therefore, we propose that backflow and transpiration serve as "overflow valves" that release internal pressure caused by phloem-derived water, thus facilitating sugar accumulation and protecting berries from cracking. Xylem backflow is especially important when berry transpiration is low (e.g., under conditions of high humidity) to ensure continued ripening and avoid berry cracking. We conclude that, in contrast to rainfall, irrigation (unless it is overhead irrigation) close to harvest does not increase berry size or dilute quality, but alleviates the potential of berry shrinkage and yield loss.

Funding support: USDA Northwest Center for Small Fruits Research, Chateau Ste. Michelle Distinguished Professorship, Rhone Rangers

Physio-Anatomical Adjustments of Grapevines to Drought Conditions

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The objectives of this study were to identify drought resistant cultivars by examining the physiological, anatomical, and morphological adaptations to drought and determine berry ripening characteristics of well-watered and water-stressed grapevines. Potted grapevine cultivars of Cabernet Sauvignon, Grenache, and Zinfandel were subjected to four water regimes: control (well-watered with full irrigation), two-thirds and one-third of full irrigation, and last treatment consisted of drying-down and re-watering cycles. Stomatal conductance of Zinfandel decreased significantly with increasing water stress while Grenache and Cabernet Sauvignon tended to be more stable. Cabernet Sauvignon and Zinfandel had lower water potential than Grenache in all treatments. Grenache showed higher relative water content than Zinfandel and Cabernet Sauvignon. Leaf vein density was higher in Grenache than in Cabernet Sauvignon or Zinfandel under both well-watered and water-stressed conditions. Leaf thickness decreased with increasing water stress in Zinfandel and Cabernet Sauvignon, whereas it increased in Grenache. Also, water stress reduced stomatal pore areas significantly but not stomatal density in all three cultivars. Palisade cell length of Zinfandel was very responsive to water stress while Grenache showed longer palisade cell length under water stress. Further-

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more, Grenache showed more intercellular air spaces than Cabernet Sauvignon and Zinfandel under water-stressed conditions. With respect to fruit composition, sugar content, malic acid, tartaric acid, and glucose+fructose were higher under well-watered than under water-stressed conditions. In contrast water stress increased total anthocyanins. In the context of drought tolerance, Grenache was structurally and morpho-physiologically better able to withstand water stress compared to Cabernet Sauvignon and Zinfandel.

Funding sources: NCSFR

Comparison of Two Commercial Rootstocks for Vineyard Water Use Under Field Conditions Using the Surface Renewal Method

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Due to increasing water scarcity in many viticultural regions of the world, there is a growing need for improving water use efficiency in commercial grape production. The use of drought-tolerant rootstocks has been suggested as an option to save water in arid and semiarid regions. A field study was carried out to compare ET_c measured with the surface renewal (SR) technique of field-grown Cabernet Sauvignon vines grafted on two widely used rootstocks, 110R and 420A, in a commercial vineyard in the North Coast Viticultural Region of California. An improved SR method, a bio-meteorological technique that can be used to accurately measure crop evapotranspiration (ET_c) at a vineyard scale, was paired with direct measurements of vine water stress and derived ET estimates to evaluate seasonal patterns of water use. SR reliably estimated ET_c in comparison to eddy covariance method, which is widely regarded as the reference bio-meteorological technique for measuring ET_c. Although rootstock differences in ET_c were small, vines grafted on 110R often exhibited slightly higher values of daily ET_c than vines on 420A. Rootstock differences in ET_c measured with the SR technique were significantly correlated with differences in midday leaf water potential (Ψ_{LEAF}) and stomatal conductance (G_s). At mild levels of water stress (between -1.0 and -0.8 MPa), G_s was less sensitive to changes in Ψ_{LEAF} in vines grafted on 110R than on 420A. Conversely, no rootstock differences in the G_s response were found at more severe levels of water stress (<-1.0 MPa). Consequently, the SR technique was sufficiently sensitive to detect even slight differences in daily ET_c and vine water status between rootstocks. These results indicate that the SR technique has the potential to be used to signal stress events for comparing water relations and water use in commercial winegrape production.

Funding support: NIFA-SCRI, USDA-ARS Sustainable Viticulture CRIS

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Viticulture — Water Relations Session – CONTINUED

Assessment of Vineyard Water Use in the Navarro River Watershed

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In California, water is considered to be a public trust resource that is used by important stakeholder groups including the interests of residential, agriculture, fish and wildlife, and other watershed needs. The Navarro River is an impaired water body as defined by the EPA Clean Water Act (excessive sediment and elevated water temperatures, and is also habitat for endangered species (Coho and Chinook Salmon). The watershed also contains the Anderson Valley wine-growing region, known for Pinot noir and other cool-climate winegrape varieties. Water use by vineyards is not well understood or defined. The industry has expanded by over 1,000 ha in the last 20 years. Concerns about water use and changes in the hydrology of the watershed require more accurate information of irrigation and frost protection water use. In this study, we used GIS information to delineate and show spatially where vineyards are located in the watershed and to determine the soil types on which vineyards are planted and their probable water-holding capacities. We reviewed private historic evapotranspiration data to develop reference evapotranspiration (ET_o) that was measured by an Adcon weather data system. We also measured multiple vineyard canopies to develop an average crop coefficient (K_c) for most vineyards. In this way, we were able to develop a representative water budget for Anderson Valley vineyards. We also sampled 40 vineyard blocks to determine irrigation system distribution uniformity. We surveyed grower practices (n = 22) on irrigation scheduling and frost protection to determine the actual amounts of water that most growers were using. We determined by survey where most growers source their water for irrigation and frost protection (ponds). We also determined the number of ponds and water diversions permitted by the State Water Resources Control Board. Finally, we determined the potential for further vineyard expansion using GIS data based on slope (<20%) and vegetation (already cleared of trees). We found that there are 1116 ha (2790 acres) of vineyards that use approximately 933 megaliters (759 acre feet) per year for irrigation and frost protection purposes. Most vineyard irrigation systems are very efficient, with an average distribution uniformity >90%. Potential evapotranspiration during the growing season was measured at 795 mm per year. Our average vine crop coefficient was determined to be .60, and 477 mm is the average calculated consumptive water use for most vineyards. In fact, grower surveys indicate that much less water is actually applied due to stored soil moisture and regulated deficit irrigation practices. Most vineyards apply less than 0.5 megaliters per year (about 5 acre inches of water). Using GIS to estimate potential vineyard

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expansion area, we found that there is an additional 1280 ha (3200 acres) that is <10% slope and free of trees, and 2680 ha (6700 acres) of area if open area <20% slope is considered. This study is a helpful planning tool to better understand water use by vineyards over a large area.

Funding support: Mendocino County Water Agency and the Nature Conservancy

In Search of Drought Resistance Characteristics among Grapevine Rootstocks: Insights from a Variety of Techniques Including X-Rays, Lasers, and Neutron Beams

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Water scarcity threatens vineyard production in dry growing regions of the western United States due to increasing competition for limited water supplies among agricultural, municipal, and conservation entities. Reduced water availability could force growers to use water conservation techniques, while attempting to maintain or enhance yield and fruit quality. To take full advantage of these strategies, growers require plant material that tolerates dry growing conditions. New grapevine rootstocks with improved drought resistance would provide a tool to improve vineyard water use efficiency and ultimately conserve water when paired with improved irrigation techniques. For cultivated crops, drought resistance can be defined as the ability of the plant to grow satisfactorily and maintain or enhance yield and fruit quality when exposed to periods of water deficit. We will describe characteristics of grapevine rootstocks that contribute to drought resistance and the methods/experimentation being used to elucidate the underlying physiological mechanism(s). Commercially available rootstocks and accessions of *Vitis* species originating from the arid southwestern United States are being evaluated using methods that include advanced imaging, gene expression, microscopy, and hydraulic physiological measurements. The ultimate goal of this work is to identify mechanistic physiological and genetic markers needed to breed drought-tolerant rootstocks from new parent material.

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Enology — Tannins Session (Part II)

Analysis of Tannins in Wine Based on Thermodynamics of Interaction

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Tannins are a complex group of plant-derived compounds found in many food stuffs, including red wine. Tannins are a critical component of overall red wine quality because of the astringency that they impart. Astringency is generally recognized as the sensation that results following the interaction and subsequent precipitation of tannin-salivary protein aggregates. In winemaking parlance, this sensation is often described using qualitative descriptive terms such as "soft" or "harsh," with variations in description considered to be due in part to tannin structure modification. Working under the hypothesis that the intermolecular forces (specifically hydrophobic interactions) that drive tannin interaction with other substrates are similar to that with salivary protein, a reversed-phase HPLC method was developed to measure the thermodynamics of tannin interaction on a solid, hydrophobic support. Thermodynamic parameters for interaction were calculated from tannin retention on a polymeric reversed-phase column thermally equilibrated to various temperatures from 30 to 60°C and at 280 and 520 nm. Based upon preliminary data from purified wine, seed, and skin tannin fractions varying in molecular size, differences in thermodynamic interaction were observed. Specifically, increasingly negative enthalpy values were observed with an increase in tannin size. Consistent with anecdotal information on astringency quality, and normalizing for tannin size, the strength of interaction declined in the following order: seed>skin>new wine>2-year-old wine. Given this result, this analytical approach was used to analyze Cabernet Sauvignon wines produced from various regions, vintages, and production methods.

Funding support: American Vineyard Foundation

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Enology — Tannins Session (Part II) – CONTINUED

Evaluation of Cap Temperature and Pumpover Volume on the Extraction of Phenolics during Red Wine Fermentation

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The phenolic content of red wine is responsible for the color, mouthfeel, and aging ability of a finished wine. While many fermentation parameters and winemaking techniques can affect the extraction of phenolics into a red wine fermentation, it is generally agreed that the primary factor in phenolic extraction is temperature. However, temperature is not uniform in a red wine fermentation and also is affected by cap management techniques such as pumpovers. Knowledge of how fermentation temperature affects the extraction of the different classes of wine phenolics during the course of fermentation rather than just in the finished wine would allow winemakers to maximize desired phenolics while minimizing those that are less desirable. To determine the effects of temperature and pumpover volume on phenolic extraction, 30 Cabernet Sauvignon fermentations were performed at the UC Davis winery in 30 gallon research fermenters. Each fermentation was carried out at different temperature regimes, with some fermentations proceeding isothermally (cap and must at the same temperature) and others proceeding with a temperature gradient between the cap and must. The effect of must exposure on the cap was investigated by altering the pumpover volume of the fermentations from one half volume twice per day to two volumes twice per day. All fermentations were sampled twice a day before pumpover and were analyzed by both UV-Vis spectroscopy and reversed-phase HPLC for phenolic content. The results from these experiments show that the pumpover volume had little effect on the extraction of phenolics. The temperature of the cap and must had a noticeable effect on phenolic extraction. For phenolics originating in the skin, temperature seemed to affect the rate of extraction but not the final concentration. For phenolics originating in the seeds, temperature seems to have an effect on both rate of extraction and final concentration of phenolics.

Funding support: E&J Gallo Winery

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Enology — Tannins Session (Part II) – CONTINUED

Rapid Wine Fingerprinting: Detection of Phenolics and Off-Flavor Compounds by DART Mass Spectrometry

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Wine quality is strongly dependent on those compounds deriving from grape and metabolism of yeast and bacteria. Phenols represent important compounds for wine quality and are responsible for color, astringency, and bitterness. Although the majority of the phenolic compounds in wine are grape-derived, some originate from microbial activity, causing off-flavor in the wine. *Brettanomyces bruxellensis*, for instance, is considered the yeast mainly responsible for the production of volatile phenols as well as other off-flavor compounds. Rapid monitoring of chemical composition of wine during fermentation and aging is essential to ensure wine quality and prevent economic loss. Ambient mass spectrometry techniques, such as DART (direct analysis in real time), allow rapid fingerprinting of wine compounds, including phenolics and off-flavor compounds such as 4-ethylphenol and 4-ethylguaiacol (medicinal and barnyard odors), isovaleric acid (rancid flavor), and tetrahydropyridine (mousy flavor) as well as their biosynthetic precursors. The DART source is unique in that samples, in their native state, can be directly introduced into the sampling region and directly ionized for MS detection. More than 25 wine samples were subjected to DART coupled to a high-resolution accurate mass quadrupole mass spectrometer (Q Exactive Orbitrap) for untargeted analysis of wines. Wine samples were analyzed directly or concentrated by sorptive stir-bar extraction using PDMS Twisters. Phenolic and off-flavor compounds in wines were identified based on their accurate masses, isotopic patterns and with an in-house database. The simple and short analysis time with DART-MS (30 sec/sample) allows a high sample throughput, enabling representative sampling of wine batches for better control of the wine production process to prevent product loss.

Funding support: IonSense, Inc.

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Industry

Collaborative Tests of ELISA Methods for the Determination of Proteinaceous Fining Agents in Wine

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Methods are needed for detecting allergenic proteins in wine after fining processes, both for legal requirements and to estimate any risk for allergic individuals. Two of the critical allergens in this case are the protein fraction from egg white and caseins. Requirements for methods were set up both by the International Organisation of Vine and Wine (OIV) and the European Commission and include collaborative tests to determine repeatability, reproducibility, limit of detection, and recovery. Two collaborative tests with 18 participants from throughout the world were conducted using commercial ELISA kits to quantify egg white protein and casein. For each analyte, nine red and white wines with different analyte concentrations were prepared and sent to the participants. Resulting sets of data were analyzed according to the AOAC by eliminating outliers and calculating the performance statistics for each assay. For wines with no or very low analyte concentrations, the values were extrapolated from the calibration curve and the resulting limits of detection are in accordance with the performance criteria set by the OIV method OIV-MA-AS315-23: R2012. For casein in white wines and egg white protein in red wines, all HORRAT values for samples with concentrations above the required limit of quantification (0.5 mg/L) were below 2. While the recovery of egg white protein from red wine is 90% or more, the recovery of casein from white wines is between 73 and 76%, which is attributable to the instability/solubility of casein in wine.

Funding support: R-Biopharm AG

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

Industry – CONTINUED

The Use of NMR in Wine Quality Control

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High-resolution NMR spectroscopy offers unique screening capabilities for food quality and safety by combining nontargeted and targeted screening in one analysis. The objective of this contribution is to demonstrate that, due to its extreme reproducibility, NMR can detect the smallest changes in concentrations of many components in a mixture, which is best monitored by statistical evaluation, yet also delivers reliable quantification results. The methodology typically uses a 400 MHz high-resolution instrument under full automation after minimized sample preparation. One analysis in a push-button operation takes ~15 minutes and delivers a multitude of results, which are automatically summarized. The method has been proven on fruit juices, where unknown frauds could be detected, be it addition of sugars and amino acids to fake 100% fruit content or mixing of different varieties, and on incorrect product labeling such as geographical origin and direct juice versus rediluted concentrate as well as on wine. The methodology developed is now transferred to wine-quality control. The advantage of NMR is its reproducibility and transferability from instrument to instrument. After setting up standard operation procedures it is possible to perform targeted analysis (quantification) on a multitude of organic ingredients (currently >50 are available) and to perform nontargeted analysis to generate information like grape variety, geographical origin, vintage year, mixing of varieties, and unexpected/unknown additives. For nontargeted screening, authentic normal models for the individual parameters have to be established, representing the normal variation of the wines under consideration. Once this is achieved, univariate and multivariate comparisons of new samples to the existing models can be performed. The possibilities of the NMR method are demonstrated on German and Italian wines, for which the first models have been built.

Funding support: Bruker BioSpin GmbH

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Enology

Thermal Inactivation of Wine Spoilage Yeasts to Validate Steam Sanitation Protocols in Wineries

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D value is the decimal reduction time or the time required to kill 90% of the target microorganism, whereas z value is the temperature (°C or °F) necessary to reduce the D value by one log cycle. D and z values are specific to strains and the menstruum where the heat inactivation is performed. Wine spoilage yeasts are primarily *Dekkera/Brettanomyces bruxellensis, Saccharomyces cerevisiae*, and *Zygosaccharomyces bailii*. D and z values of these genera were obtained to determine target thermal treatments for wine cooperage. Our findings resulted in a maximum log reduction of 3.2 at 50°C (122°F) in *Dekkera/Brettanomyces bruxellensis* populations. The highest temperature that allowed for survival of *Brettanomyces* was 55°C (131°F). The temperatures used to determine D and z values for these strains were 45, 50, 52.5, and 55°C. With regard to *S. cerevisiae* strains, the highest log reduction was 4 at a temperature of 50°C. The temperatures used for these strains were 45, 50, 52.5, 55, 57.5, and 60°C. In contrast, *Z. bailii* had a maximum log reduction of 3.4 at 50°C; the temperatures used for this strain were 50, 55, and 57.5°C. A post hoc study was done using steam (validation method) with naturally contaminated barrels where the thermal penetration was monitored at two different times (5 and 10 min) and depths (8 and 14 mm). Using a 10-min exposure and a depth of 8 mm, the highest temperature reached was 57.5°C, consistently taking 10 min and 4 sec; however, 14 mm depth reached a maximum temperature of 42.5°C. These findings suggest that a minimum of 10 minutes is necessary to consistently reach temperatures to kill the target wine spoilage microorganisms used in this study.

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Peroxyacetic Acid: Sanitizing Efficacy on Wine Barrels

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Peroxyacetic acid is a common sanitizer used in the food, beverage, and wine industries. Its effectiveness as a sanitizer for wine barrels has not been reported. The purpose of this study was the evaluation and validation of this sanitizer at two different concentrations (120 and 60 mg/L) using seven wine spoilage strains corresponding to the genera *Brettanomyces bruxellensis, Saccharomyces cerevisiae*, and *Zygosaccharomyces bailii*. At a concentration of 120 mg/L, all *Brettanomyces* strains tested (CE261, CE149, and 2080) were reduced below detectable limits almost immediately after exposure. However, at 60 mg/L, CE261 was reduced to below detectable levels after 5 minutes. The other

*indicates corresponding author

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strains (2080 and CE149) were reduced to below detectable levels almost immediately after exposure. When *Saccharomyces* strains (CE81, CE78, and CE9) were exposed to 120 mg/L, all of them were reduced to below detectable levels; however, CE78 was reduced after 5 min and CE9 and CE81 were reduced almost immediately after exposure. Furthermore, 5, 1.8, and 6 log reductions were found when 60 mg/L were used for strains CE81, CE78, and CE9, respectively. These results suggest that with *S. cerevisiae* strains, the level of reduction is strain and concentration dependent. Finally, when *Z. bailii* was challenged with 120 and 60 mg/L, only 4.3 and 0.45 log reductions were achieved, respectively, suggesting that of the three genera used in this experiment, *Z. bailii* is highly resistant to the concentrations used. The highest concentration was used to test the effectiveness of peroxyacetic acid in naturally contaminated barrels. However, 120 mg/L was not as effective for the porous surface of wine barrels, leaving detectable levels of general yeast populations and *Brettanomyces.* Alternatively, 200 mg/L and one week exposure time were used to sanitize the barrels and no detectable levels of microorganisms were found afterward.

Funding support: CONACYT-MEXICO, research program funds, and a research travel grant from Cornell University 2012

Sanitation of Wine Cooperage Using Five Different Treatment Methods: An In Vivo Study

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Critical evaluation of decontamination treatments is crucial to determining efficacy of sanitizers, especially for porous surfaces of wooden wine barrels. Furthermore, efficacy must be determined against spoilage microorganisms relevant to wine production. We compared efficacy of five sanitation treatments (sulfur dioxide, peroxyacetic acid, steam, chlorine dioxide, and ozone) in 100 barrels contaminated by populations of general yeasts and *Brettanomyces*. Sulfur dioxide discs (5 g) were applied and held for 3 or 6 weeks. The 6-week treatment reduced both yeast populations in the majority of the barrels below detectable levels. The 3-week treatment reduced *Brettanomyces* to undetectable levels in all the barrels sampled, but was not as effective in eliminating the general yeast population. Peroxyacetic acid was applied at 200 or 120 mg/L, respectively. The 200 mg/L treatment reduced all populations to undetectable levels, but populations remained detectable after 120 mg/L treatments. Steam applied for 5 or 10 min reduced *Brettanomyces* populations to undetectable levels; however, detectable levels of general yeast populations were found in both times. The degree of heat penetration into the staves from steam was determined at two different depths. Chlorine dioxide yielded no

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or slight reduction (1 log) for both yeast populations with the concentrations tested (5 or 10 mg/L). Ozone (1 mg/L) applied for 5 and 10 min reduced both *Brettanomyces* and general yeasts populations to undetectable levels in most barrels. Alternatively, wood cores were obtained from the staves (pre- and postsanitation treatments) in order to corroborate *Brettanomyces* presence in the pores of the wood. Mostly, *Brettanomyces* isolates were found in pretreatment cores. Our study revealed wide variation in efficacy of commonly used decontamination treatments and provides guidance to winemakers in ranking the relative efficacy of methods used to eliminate spoilage microorganisms in wine cooperage.

Funding support: New York State Agricultural Experiment Station Federal Formula Funds, Constellation Brands, and CONACYT-MEXICO

Contributions of Grape Berry Compounds to Wine Aroma

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Aroma compounds have major influences on consumer preferences for wine. This has led to interest in understanding the development of wine aroma compounds and variables affecting the final volatile composition of wines. Understanding the origin of important wine aroma compounds could allow interventions in grapegrowing and wine production that improve characteristics that are desired by wine consumers. Grapes contribute to the composition of final wine, not only through varietal impact compounds such as terpenes and methoxypyrazines, but also through nonvarietal aroma compounds, such as C_6-alcohols and some esters of higher alcohols. A recent study in our group showed that many aroma compounds increased in model ferments as the proportion of Riesling or Cabernet Sauvignon grape juices increased. This presentation outlines further investigations that have been undertaken to explore the origin of aroma compounds that are dependent on grape composition. This work has taken a natural products separation approach and aimed to purify compounds in grapes that influence the production of volatile compounds in wines. Results will be presented on our studies into the production of ethyl esters and lactones in wines as influenced by purified fractions from ripe grapes. These results will be discussed in terms of the mechanisms by which the precursors may be determining the concentrations of these common volatile components of wines.

Funding support: Commonwealth Scientific and Industrial Research Organisation (Plant Industry) and The Grape and Wine Research and Development Corporation

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Enology – CONTINUED

Non-*Saccharomyces* Strains: Interactions with Anthocyanins uring Tannat Wine Fermentation

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Yeast strain selection was demonstrated to be important for color preservation during red wine vinification by *Saccharomyces cerevisiae*. Limited information was reported about yeast-mediated changes by non-*Saccharomyces* strains in wine pigment composition. In this work, anthocyanin and derived compound concentration during red wine fermentation was studied with the objective of classifying yeast strains according to their capacity for color modification. A model red grape juice medium without grape solids containing Tannat polyphenol compounds was used to better understand yeast-anthocyanin interactions. Fifty selected non-*Saccharomyces* strains, belonging to the genera *Metschnikowia, Hanseniaspora, Candida, Torulospora,* and *Issatchenkia* were screened for their effect in wine color. The results showed that strains *Metschnikowia pulcherrima* M00/09G, *Hanseniaspora guillermondii* T06/09G, *Hanseniaspora opuntiae* T08/01G, *Hanseniaspora vineae* T02/5F, *and Hanseniaspora clermontiae* (A10/82F and C10/54F) presented the best color performances compared with *Saccharomyces cerevisiae*. According to this data, anthocyanin derivative pigment formation was studied by HPLC-MS, while the quantification of several derivatives was performed by HPLC-DAD. The formation of vitisin A and B and other pyranoanthocyanins by these strains was identified in single culture treatments. The production of these stable pigments made a significant contribution by mixed culture fermentations by comparison with single cultures of conventional commercial yeast strains. Further studies at winemaking level would be necessary to prove the potential of non-*Saccharomyces* strains to increase red wine color and aging potential of Tannat wines.

Funding support: Agencia Nacional de Investigacion e Innovacion, Uruguay; Universidad de la Republica Faculty of Chemistry; CSIC Group UdelaR

Elucidation of Pigmented Tannin Structure and Importance in Red Wine Color by High-Resolution Mass Spectrometry

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As pigmented tannin is responsible for persistent color in red wine, it is important to understand its complete role in red wine color and sensory perception. It is well known that the effects due to copigmentation and monomeric antho-

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cyanin disappear within the first several years of red wine aging, yet a bold red color may remain in wine that is decades old. With this study, fundamental information on the structure of these wine pigments is being evaluated using matrix-assisted laser desorption ionization and electrospray ionization coupled with Fourier transform ion cyclotron resonance mass spectrometry. A comprehensive evaluation of the polymeric constituency of red wines revealed potential compounds previously unknown. These methods have revealed exact mass and thereby molecular formulas. Structural evaluation continues by means of quadrupole–time of flight and triple quadrupole (QQQ) tandem mass spectrometry (MS), using isolated ion fragmentation to obtain structural information. The goal of this project is quantitative determination of the extent of color due to the polymeric composition of the wine matrix by using a suite of complementary mass spectrometric methods and absorbance spectroscopy. Implications of this methodology include evaluation of the reactions involved in tannin cross-linking as well as the folding of large polymeric constituents in aged wines leading to altered sensory perception.

Funding support: American Viticulture Foundation

Aroma Compounds in CO_2 Wine Fermentation Flow

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The volatile compounds that are lost during the fermentation of must into wine represent a part of the winemaking process. The capturing and analysis of the volatile compounds that are purged with the carbon dioxide (CO_2) flow during gas exhaustion of the fermentation stage are the main objective of the present study. The volatile compounds purged with the escaping CO_2 flow were captured using solid-phase extraction (SPE) cartridges attached to the airlocks of the fermentation vessels. *Tamaioasa romaneasca* (a traditional Romanian variety) grapes were used as biological material. Extracts of retained volatiles were obtained by eluting the polymeric sorbent of the SPE cartridges with dichloromethane and analyzed by gas chromatography coupled to mass spectrometry (GC–MS) to identify and quantify the captured compounds. With respect to the total amount, highest losses occurred with low molecular weight alcohols, esters, and acetic acid. There were also losses for other volatile compounds, such as terpenoides, including linalool. Such compounds usually are found in trace quantities but, due to their often low flavor threshold values, they may contribute to the perceived aroma.

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Sequential Inoculation of Non-*Saccharomyces* in Riesling Vinification

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Increasingly, winemakers are seeking for ways to introduce aroma and flavor diversity to their wines as a means of improving style and increasing product differentiation. In this context, spontaneously fermented wines or fermentations inoculated with non-*Saccharomyces* yeast are becoming very popular. This study aims to evaluate the performance of selected commercial non-*Saccharomyces* yeast on wine aroma in sequential inoculation. The yeast *Torulaspora delbrückii, Metschnikowia pulcherima, Pichia klyveri, Kloeckera apiculata*, and a combination of them were used to perform fermentations of Riesling grapes harvested in 2012. The fermentations were performed in 50 L pilot steel tanks and the yeast inoculations were performed according the indications of the provider. The experiment included several natural spontaneous fermentations, fermentation with pure cultures of *Saccharomyces* yeasts, and inoculation with pure cultures of non-*Saccharomyces* yeasts. To simulate natural spontaneous fermentation with delayed dominance of non-*Saccharomyces* and *Saccharomyces* yeasts, several vinifications with sequential inoculation of pure cultures of non-*Saccharomyces* yeasts and pure cultures of *Saccharomyces* yeast (called imitated spontaneous fermentation) were performed. Aroma composition using headspace gas chromatography–mass spectrometry (HS/GC-MS), chemical parameters (pH, titratable acidity, and levels of residual glucose), organic acids (acetic, lactic, malic, and tartaric), phenolics (total phenolics, tannins, and anthocyanins) are currently being studied for assessment of fermentation performance.

Funding support: Rheinland-Pfalz Government Fraunhofer Institute IME

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Use of Non-*Saccharomyces* Yeast as a Novel Source of Mannoproteins in Wine

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Yeast-derived mannoproteins have been shown to reduce wine astringency, increase wine body and smoothness, and achieve a natural stabilization of both wine protein and tartrate. Commercial mannoprotein-rich preparations are available and are currently being used in winemaking to obtain desired organoleptic and stability effects. In order to increase the amount of mannoproteins released during the alcoholic fermentation, researchers have evaluated genetically engineered wine yeast strains or autolytic thermosensitive mutants of *Saccharomyces cerevisiae.* Recent studies have revealed that some non-*Saccharomyces* wine yeast strains show a much higher capacity to release polysaccharides and mannoproteins compared to *S. cerevisiae* strains. In the present work we evaluated the ability of eight non-*Saccharomyces* strains to release mannoproteins. These yeasts, belonging to different genera, have been isolated from grape and must of different origins, and were selected for their enological aptitudes in mixed fermentation at the laboratory scale. The fermentations were carried out in synthetic media simulating grape juice using the non-*Saccharomyces* yeast in pure culture. Quantification and chemical composition of the glycoproteins released during the alcoholic fermentation was performed by HPLC and MALDI-TOF (matrix-assisted laser desorption/ionization time of flight). The results showed a consistent increase of the polysaccharide level released into the media from the non-*Saccharomyces* yeast strains compared to that obtained from a commercial *S. cerevisiae* strain, under the same conditions. A different chemical composition of the sugar moieties and protein percentage of the glycoproteins was also observed. The possibility of increasing naturally the content of polysaccharides by judicious use of non-*Saccharomyces* yeast during the alcoholic fermentation represents an additional option for organoleptic modification of wines.

Funding support: Dipartimento di Gestione Sistemi Agrari, Alimentari e Forestali, Università degli Studi di Firenze

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Yeast Dynamics during Inoculated and Spontaneous Fermentations of Two Different Vintages at a British Columbia Winery

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In both inoculated and spontaneous fermentations, recent evidence supports the idea that the yeast community fermenting wine can be highly influenced by the yeast residents of the winery. The objective of this study was to compare yeast dynamics during inoculated and spontaneous fermentations from two vintages produced at a single Canadian winery. In addition, a comparison of the yeast assemblage in fermentation tanks with yeast inoculants used by the winery was made to determine the influence of resident yeasts on the yeasts fermenting the wine. During the 2011 and 2012 vintages, three inoculated and three spontaneous *Vitis vinifera* L. var. Pinot noir fermentations from Quails' Gate Estate Winery were sampled from four stages (cold soak, early, middle, and end). *Saccharomyces cerevisiae* isolates were discriminated at the strain level by microsatellite analysis and identified by comparing DNA fingerprints to microsatellite databases. Non-*Saccharomyces* species were identified by sequencing the D1/D2 domain regions of rDNA. For the two vintages, implantation of the inoculant ranged from 56 to 100% at the end of fermentation. In the early, middle, and end stages of both inoculated and spontaneous fermentations, the predominant yeasts were either inoculated or non-inoculated commercial ADY strains previously used at the winery. For all fermentations, a typical succession from non-*Saccharomyces* to *Saccharomyces cerevisiae* strains was observed. On the whole, the composition and diversity of yeast assemblages were similar for the two different vintages. Yeast assemblages developing after cold soak were similar to the strains used at the winery. Data, such as these presented in this study, are useful for winemakers when it comes to making yeast selections for their fermentations.

Funding support: Natural Sciences and Engineering Research Council of Canada; Quail's Gate Estate Winery

Metabolism of Hydroxycinnamic Acids and Their Tartaric Acid Esters by *Brettanomyces* and *Pediococcus* in Red Wines

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Caffeic, *p*-coumaric, and ferulic acids and their corresponding tartaric acid esters (caftaric, coutaric, and fertaric, respectively) are found in wines in varying concentrations. While *Brettanomyces* and *Pediococcus* can utilize the free acids, it is not known whether they can metabolize the corresponding tartaric acid esters. Syrah, Pinot noir, Cabernet Sauvignon, and Merlot wines were

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inoculated with one of four strains of *Brettanomyces bruxellensis* (B5, I1a, E1, or B1b) at 10^5–10^6 cfu/mL. In a second experiment, Pinot noir and Syrah wines were inoculated with either strain of *Pediococcus parvulus* (WS 7C or WS 29A) at 10^6–10^7 cfu/mL, and after two weeks, half of the wines were inoculated with *B. bruxellensis* E1 at 10^5 cfu/mL. Wine samples were fractioned by C_{18} solid-phase extraction columns with caffeic, caftaric, *p*-coumaric, coutaric, and ferulic acids analyzed by HPLC-DAD. *B. bruxellensis* B5 populations declined to less than 30 cfu/mL and metabolized 12% or less of the caffeic, *p*-coumaric, and ferulic acids. Conversely, strains I1a, E1, and B1b entered exponential growth and reached populations in excess of 10^7 cfu/mL. Additionally, the three strains metabolized 43–71% of the caffeic acid, 87–98% of the *p*-coumaric acid, and 37–78% of the ferulic acid. However, strain I1a exponential growth and the onset of the free hydroxycinnamic acids utilization were delayed one to three weeks compared to strains E1 and B1b. None of the *B. bruxellensis* strains were able to metabolize the hydroxycinnamic acid tartaric acids esters. *P. parvulus* WS 7C and WS 29A populations slowly declined one log. Both strains were able to convert 25–38% of the caffeic acid and 13–20% of the *p*-coumaric acid. Neither strain of *P. parvulus* utilized the ferulic acid or hydrolyzed the hydroxycinnamic acid tartaric acid esters. Both *B. bruxellensis* and *P. parvulus* demonstrated strain and wine dependent metabolism of the free hydroxycinnamic acids.

Funding support: Washington Wine Advisory Committee

Influence of Barrel Maturation, Oak Alternatives, and Microoxygenation on Red Wine Aging and Quality

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The impact of storing red wine in stainless-steel containers with microoxygenation in conjunction with a variety of oak alternatives on phenolic composition and wine aging was investigated and compared with traditional barrel aging. A blended red wine was aged in new French and American oak barrels as well as in sealed stainless-steel containers, which were equipped with a microoxygenation system and either oak chips, oak staves, or oak tannin extracts. Oxygen was administered at a rate of 1 mg/L per month in order to emulate the ingress of oxygen occurring in the wood barrels. After 6 months of aging, all wines were bottled and analyzed. Differences in the extent and composition of polymerization reaction products were determined via phloroglucinolysis and LC–MS analyses, and total phenolic content was characterized by both UV-vis and RP-HPLC. Differences in color formation, likely due in part to the catalytic effect of oxygen and ellagic tannins on indirect polymerization reactions, were observed between different treatments. After three months of

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bottle aging, all wines were assessed sensorially via a descriptive analysis panel, with an emphasis placed on distinguishing differences in mouthfeel characteristics. Preliminary results indicate that microoxygenation and tannin additions both had a positive impact on enhancing the perception of smoothness in treated wines, but less so than traditional barrel aging.

Funding support: American Vineyard Foundation

Lingering of Wine: A Methodological Approach to a Complex Sensory Incidence in a Connoisseur's Oral Laboratory

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Comments about wine often refer to lingering sensory modalities such as sweetness or bitterness. Time intensity (TI) analysis measures the rise and decline of orally perceived attributes one at a time. Temporal dominance of sensation analysis was developed to replace the time-consuming TI method by recording over time how many panel members perceived an attribute as the most dominating one from a given set. Thus, perception of intensity in TI methodology was traded for frequency of choice in TDS analysis. A special focus of this study dealt with bitterness, as bitterness is a very persistent sensation, leading at least in white wines most often to negative connotation by consumers. However, the molecular base for bitter taste in white wines is still widely unknown. In a first step, 13 commercial white wines were evaluated by the same panel using TI, TDS, and standard descriptive analysis (DA). To facilitate a statistical comparison, parameters were extracted from bitterness curves obtained from TI and TDS and were correlated with bitter intensities and bitter persistency recorded by DA. Analysis of variance not only revealed statistical differences among the wines for each bitter parameter extracted from all techniques, but also provided specific information, how they are correlated with each other and with major wine constituents. While bitterness parameters of TI analysis were mostly driven by ethanol content, TDS parameters and bitter intensity and persistency assessed by DA were related to the masking effect of residual sugar. In a second step, white winemaking was varied by duration of skin maceration, solid contents, yeast strains, and lees contact. Based on TDS parameters obtained from each repetition, for each attribute an ANOVA was conducted. This approach proved to be useful to test the statistical significance of sensory differences among the experimental wines, based on their temporal dominance.

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Inline Dielectric Sensor for Real-Time Monitoring of Yeast Growth in White Wine Fermentation

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An inline dielectric flow cell sensor has been developed to monitor the population of yeast, in real time, during the early phases of white wine fermentation. The sensor's measurement technique quantifies the complex dielectric permittivity spectrum of wine while in the presence of grape particulates and bubbles. The flow cell sensor was tested during the fall 2012 harvest at the UC Davis Robert Mondavi Winery on three Vermentino fermentations performed at 15°C. The measurements were made inline over approximately 50 hours, corresponding to the yeast growth phase, in the 300 kHz to 100 MHz range. The termination of the growth phase was determined by monitoring the decline of Brix through an inline measurement. During fermentation, independent verifications of cell growth were performed by methods that include light scattering and hemacytometry. These initial experiments obtained a correlation R^2 of at least 0.92. Experimental trials successfully demonstrated the ability of the sensor to detect critical population inflection during the first days of fermentation.

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Automation of Fed-Batch Vinifications by FT-NIR Spectroscopy: Effects on Yeast Metabolism and Fermentation Dynamics

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During the batch fermentation of wines, high sugar concentrations present in musts from late harvest or hot climate grapes elicit a hyperosmotic stress response in yeast, increasing the risk of sluggish or stuck fermentations and causing elevated formation of undesired by-products. Climate change mediated increase in grape sugar content has further exacerbated this problem. To address these challenges, we used a previously developed FT–NIR spectroscopy-based system that allows for continuous monitoring of glucose, fructose, total sugars, and ethanol levels in actively fermenting wine to conduct fully automated fed-batch vinifications, where must is slowly added to an active fermentation to maintain constant low sugar concentrations. Calibrations were created using wine standards and achieved highly satisfactory predictions for all analytes ($R^2 > 93$). When used to maintain a test fed-batch fermentation

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at a target sugar concentration of 45 g/L, the system performed well, keeping sugar levels within 5 g/L of the set point. By fermenting under automatically sustained low Brix conditions, the described system prevents the osmotic stress response and causes dramatic (50 to 90%) reductions in the final concentrations of glycerol, acetic acid, and acetaldehyde, an increase in viable yeast numbers, and changes in the formation of aroma relevant esters, as compared to a traditional batch fermentation. The fed-batch platform also allows fermenting wine to reach 90% of its theoretical alcohol content after adding only 10% of the must. Ethanol concentration then remains essentially constant due to the balancing rates of formation and dilution. This continuous culture-type system significantly reduces the risk of microbial contamination during fermentation since the majority of the must is added to a high alcohol wine. While the cost of spectroscopic instrumentation is decreasing, our studies suggest that with some minor adjustments, more affordable analytical tools such as hydrometers or the Clinitest assay can also be used to enable fed-batch fermentations.

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An Optimized Method for the Measurement of Carbonyl Compounds in Wine by Liquid Chromatography–Mass Spectrometry

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Carbonyl compounds are produced from the chemical oxidation of major wine components during winemaking and aging, and they are important to wine flavor and color stability. As these compounds can react reversibly with bisulfite to form α-hydroxysulfonates, alkaline treatment has been used in a previous published method prior to derivatize with 2,4-dinitrophenylhydrazine (DNPH) to increase the concentration of carbonyls. To simplify the analysis of carbonyls, we have developed an optimized method without alkaline treatment by liquid chromatography–mass spectrometry (LC–MS). After wine sample acidification, carbonyl compounds are derivatized with DNPH in less time. The reaction is optimized for temperature, time, and amount of acidification reagent. Oxygen exclusion of reagents is necessary. This method for acetaldehyde, 2-ketoglutaric acid, and pyruvic acid analysis offers good specificity, high recovery, and low limits of detection. It was successfully used to monitor concentration changes of carbonyl compounds in different wines. Method validation for other carbonyl compounds is currently in progress.

Funding support: University of California, Davis

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Impact of Grape Sugars on Combined Assay for Protein Precipitable Tannins and Polymeric Pigments

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A protein precipitation assay for tannins has been adapted for use with grapes and when utilized on wine it was found that it could also be used to separate polymeric pigments into two classes (small, SPP; large, LPP) designated by their ability to precipitate protein. As this analytical approach has been utilized in the wine industry, the extent of variation in the tannin analysis caused by altering the wine matrix has largely gone unevaluated. Sugar and ethanol are the most abundant compounds in wine and are known to vary significantly during the winemaking process. The effect of ethanol has been evaluated and it is known that in the range found in wine there is no significant impact on the analysis. For this set of experiments various different sugars found in wine were evaluated (glucose, fructose) including sucrose, which is not. Wines were modified with increasing sugar concentrations and decreasing tannin concentrations and assayed. Increasing sugar concentration decreased the precipitation of tannin and LPP. The use of a hydrogen bond disruptor (urea) to reduce protein–tannin and protein–pigment complex formation showed that the effect of sugar concentration occurred by increasing the solubility of the tannin–protein complex, not by interfering with protein–tannin complex formation. By increasing the solubility of pigment–protein complexes, SPP appeared to increase. The total polymeric pigments also increased at each tannin concentration with increasing glucose and sucrose concentration, indicating that sugar concentration might also affect bisulfite bleaching of anthocyanins. While a significant effect of sugar concentration on tannin–protein complex solubility was observed, these effects were greatest at sugar concentrations far in excess of normal winemaking conditions. Under normal winemaking conditions, sugar concentration will have a negligible effect on protein-precipitable tannin, LPP, and SPP concentrations.

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Wine Aroma: Identifying Key Sensory Attributes for Emotional Responses

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In competitive wine markets, companies strive to identify factors influencing consumer perception of wines. Evaluating wine attributes that include odor-elicited emotional responses may provide insight into consumer purchases. Consumer and descriptive sensory analysis were used to determine emotional responses to wine odors and overall liking, identify wine odor attributes, and determine drivers of emotional responses for wine odors. A commercial white wine was used for the five wine treatments: control, acetaldehyde-spiked, diacetyl-spiked, linalool-spiked, and 2,4,6-tricholoranisole (TCA)-spiked. In the consumer study (n = 89), emotional responses were elicited by odors for 33 of the 36 terms using the Geneva Emotion and Odor Scale (GEOS). Emotional responses were elicited for the major dimensions of GEOS (disgust/irritation, happiness/well-being, sensuality/desire, energy, soothing/peacefulness, and sensory pleasure). Linalool-spiked wine and the control wine elicited more happiness/well-being, energy, and sensory pleasure than TCA-spiked wine. Linalool-spiked wine elicited more sensuality/desire than TCA-spiked wine. The TCA-spiked wine elicited more disgust/irritation and less soothing/peacefulness than the other wines. For overall liking of wine odors using a nine-point hedonic scale, linalool-spiked wine (6.4) scored higher than acetaldehyde-spiked (6.0), diacetyl-spiked (6.0), control (5.8), and TCA-spiked (4.6). Ten descriptive panelists identified and scaled 24 odor attributes. Significant wine odors included sulfur, moldy/musty, caramelized, honey, leather, and dirty socks. TCA-spiked wine had the highest moldy/musty and leather odor. Diacetyl-spiked wine had the highest caramelized and honey odors. Moldy/musty wine odor was negatively correlated to overall liking ($r = -0.92$) and positively correlated to disgust/irritation emotion ($r = 0.89$). Overall liking of wine odor was negatively correlated to disgust/irritation ($r = -0.97$) and positively correlated to happiness/well-being, sensuality/desire, energy, soothing/peacefulness, and sensory pleasure ($r > 0.92$). Partial least squares regression supported the correlation results. Measuring odor-elicited emotional responses in addition to traditional consumer and descriptive analysis can provide a deeper understanding of the consumer perception of wines.

Funding support: University of Arkansas Department of Food Science

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The Effect of Dealcoholization on the Sensory Profiles and Consumer Preference of White Wine

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The alcohol content of U.S. wines has risen dramatically in recent years, leading to financial and social implications. One way to combat this is dealcoholization—the removal of alcohol from wine. Little is known about how changes in alcohol levels affect wine sensory compositions. The aim of the study was to investigate the influence of dealcoholization on wine sensory profiles and determine whether consumers can perceive changes in wine alcohol levels. California oaked 2010 Chardonnay with 14.8% v/v alcohol was dealcoholized and blended back to the original wine at 0.2% v/v increments from 12.8% v/v to the original alcohol level. Consumers (n = 101) assessed six wines spanning a 1% v/v range for liking and sorting. Descriptive sensory analysis and difference testing were conducted on the wines using trained panelists. There were no differences in consumer liking, indicating that consumers were unable to detect changes in alcohol levels less than 1% v/v. Overall, consumers were unfamiliar with the dealcoholization process and were significantly less likely to purchase reduced alcohol white wine at any price point. In the descriptive sensory analysis, where the minimum alcohol difference between wines in one session was 0.4% v/v, wines differed ($p < 0.07$) for *floral* and *butter* aromas and *hot* mouthfeel. A triangle test using wines with at least 1% v/v difference showed that panelists were unable to correctly identify the different samples. The same samples were also assessed using a directed, paired comparison, where panelists were asked to identify the sample highest in alcohol. Panelists were able to differentiate between samples, indicating that at a 1% v/v or greater difference, wines clearly differed based on *alcohol* aroma and *hot* mouthfeel. The results of this study suggest that the reduced alcohol wines were very similar in composition to the original wine, indicating that dealcoholization of up to 2% v/v in oaked Chardonnay does not overly impact wine sensory composition.

Funding support: J. Lohr Vineyards & Wines and Constellation Brands, Inc.

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Assessing Effects of Bottle Pressure on Volatile Sensory Profiles and Bubble Nucleation of Sparkling Wine

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The sensory properties of sparkling wines are driven not only by chemical compounds but also by the amount of CO_2 and pressure in the bottle. We were interested in characterizing the sensory attributes of Californian blanc de blanc sparkling wine as well as the sensory impact of artificial nucleation points on the aroma and flavor. The sensory profiles of eight California blanc de blanc were determined using a generic descriptive analysis. Eleven trained judges created, were trained in, and used visual, aroma, taste, mouthfeel, and bubble descriptors to characterize the sensory differences among the sparkling wines. A potential temporal effect on the aroma and flavor development of California blanc de blanc was determined by performing the descriptive analysis 1 minute and 5 minutes after pouring, using the same descriptors. In addition, the atmospheric pressure of each wine bottle and the volatile profiles of all wines were measured to determine the influence of pressure on taste and aroma over time. As a second research question, the effect of artificial nucleation points on the aroma and flavor intensity was studied. For this, three different nucleation treatments (air-dried glasses, paper towel dried glasses, and glasses with an artificial nucleation point) were compared to "natural" nucleation points (normal glasses), and 33 panelists assessed overall aroma and flavor intensity for each treatment. For the nucleation study, we could not find any statistically significant differences in aroma and flavor intensity. For the descriptive analysis on the California blanc de blanc wines, significant differences were found for visual, aroma, taste, and mouthfeel descriptors.

Funding support: none

Judging Wine Quality: Do We Need Experts, Consumers, or Trained Panelists?

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Twenty-seven Cabernet Sauvignon wines from all Californian wine regions were selected for this study based on their performance in the 2012 California State Fair Wine Judgement. For each of the nine regions, we selected wines with high, medium, and low ratings, representing high, medium, and low wine quality, as determined by the State Fair wine experts. A descriptive analysis

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(DA) on these wines was conducted with 15 trained judges, who evaluated differences among the wines using 21 aroma, three taste, and three mouthfeel descriptors. Color differences were assessed holistically using a sorting exercise in which the judges grouped the wines according to similarity of color. One hundred and seventy-four consumers were recruited for a hedonic assessment of the wines, using a balanced incomplete block design. Each consumer tasted six wines and rated overall liking and overall quality. Demographics (age, sex, income), wine consumption, and wine expertise were obtained from each consumer. Overall, trained judges found that the wines differed significantly in aroma, taste, and mouthfeel properties. Wines showed significantly different sensory profiles with some correlation to the assigned quality scores; for example, most of the low-quality wines were characterized by one particular attribute (for example, sulfur or chemical), while high-quality wines typically showed a more balanced profile. Significant differences in liking were found for the consumers, which segmented into four clusters. Some of the clusters could be related to demographic information such as wine consumption and wine expertise. Consumers did not agree completely with the assigned quality categories and gave high liking scores to all three quality categories.

Funding support: American Vineyard Foundation

Effect of Storage Temperature and Packaging Type on the Trace Metal Analysis of Wine

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The determination of shelf life after bottling or filling is a crucial aspect for determining final wine quality. Storage temperature and packaging type can have important impacts on wine shelf life. In this study, California Chardonnay (vintage 2010) was stored at three different constant temperatures (10, 20, and 40°C) for three months in four different packaging configurations: 0.75 L glass bottles with ROTE screwcaps, natural corks, or synthetic corks, and 3 L bag-in-boxes. After storage, all samples were analyzed using a generic descriptive analysis for aroma, flavor, taste, and color by 14 trained panelists. Panelists could sense a metallic taste in some of the wine samples. To determine which storage conditions and package types led to the metallic taste, each wine was tested for trace metal contaminates using an Agilent 7700x ICPMS. Over 20 metals were tested and results showed that tin was leaching into the wine under some of the conditions tested. Additional studies were conducted to determine the origin of the tin from the different packaging types used in this study.

Funding support: Wines and synthetic corks donated by Constellation Brands; natural corks donated by ACI Cork USA, Inc.

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Isolation and Properties of the Bacteria Associated with Deacidification in Campbell Early Must

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The grape cultivar Campbell Early has high levels of malic acid as well as tartaric acid. The high concentration of total acid in the Campbell Early wine is a critical aspect of the wine's sensory characteristics. To prevent the deterioration of the wine's quality, which is caused by the strong sour taste derived from the raw material in winemaking, the deacidification factor was investigated via carbonic maceration under different temperature conditions, especially in the presence or absence of malolactic bacteria. Based on the results of the presence test of the malolactic bacteria during carbonic maceration treatment, *Lactobacillus brevis, Lactobacillus plantarum,* and *Streptococcus thermophilus* were characterized morphologically and were identified via biochemical tests and 16S rRNA gene-sequencing analysis. The isolated strains were found not to consume malic acid and to produce lactic acid. Moreover, these strains were consumed as soluble solids. The isolated strains are popularly known as lactic acid bacteria and should have produced lactic acid from glucose. The *Oenococcus oeni* of the malolactic bacteria was not isolated. These results showed that the isolated strains are not deacidified during carbonic maceration treatment.

Funding support: Next-Generation BioGreen 21 program (grant PJ008213032012), Rural Development Administration, Republic of Korea

Evaluation of Qvevri in Winemaking

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Qvevri were the primary earthenware vessels used for the fermentation and storage of wine originating in the Caucasus around 8,000 BCE. Recent recognition and appreciation of Georgian wine, wine history, and winemaking techniques are leading to newfound interest in Georgian winemaking traditions and technology, including qvevri, beyond its borders. There are few reports on effects to a wine's composition after storage in qvevri. This study evaluated the porosity of qvevri and the influences of clay and beeswax on specific wine volatile compounds. Four 3 L qvevri, four 4 L glass jars with lids, beeswax, model wine, and a commercial red wine were used. Porosity was measured by the average weight change before and after soaking with water

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for 24 hours. Known concentrations of IBMP, IPMP, ethyl acetate, β-linalool, and geraniol were added to the model wine. The wine was stored in four qvevri glazed with beeswax, two glass jars glazed with beeswax, and two glass jars without bees wax. The containers were stored at 20°C and analyzed weekly for 30 days. IBMP, IPMP, ethyl acetate, β-linalool, and geraniol were quantified by SBSE GC-MS. The experiment was repeated using red wine following the same procedure. The qvevri absorbed liquid, averaging about a 1% increase in total weight after 24 hours. Significantly high concentrations of IBMP and IPMP were measured in the model wines stored in the qvevri. The clay material of the qvevri, which has higher porosity than glass, is likely responsible for the increased concentration of pyrazine compounds in the wines that may result in higher vegetal flavor intensity. Ethyl acetate, β-linalool, and geraniol concentrations in the model wines stored in qvevri were not affected by the clay. Beeswax glaze absorbed β-linalool, geraniol, IBMP, and IPMP, but did not affect ethyl acetate concentration in both model wine and red wine.

Funding support: Sleeping Dog Studio, San Marcos, Texas, donated the qvevri; Walker Honey Farms, Rogers, Texas, donated the beeswax

Comparison of Volatile Composition and Sensory Characteristics of Korean Rice Wines (Makgeolli)

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Quantitative sensory profiles of 10 commercial rice wines (Makgeolli) with heat treatment were developed using sensory descriptive analysis. Three appearance, seven aroma, seven flavor/taste, and four mouthfeel related sensory attributes were evaluated in duplicate by a panel of 10 judges. From the principal component analysis (PCA) of the descriptive data, rice wines were primarily separated along the first principal component, which accounted for 61% of the total variance between the rice wines with high intensities of white color and fruitiness versus yellow color and yeast flavor. The volatile compounds of same rice wines were extracted and analyzed by headspace solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS), respectively. A total of 50 volatile components were detected, including 24 esters, 7 alcohols, 4 acids, 3 ketones, 2 aldehydes, and 10 miscellaneous compounds. Esters and alcohols were the largest groups among the quantified volatiles. About 60% of the total quantified volatile material was contributed by six compounds: ethyl octanoate, ethyl decanoate, ethyl hexadecanoate, ethyl dodecanoate, 3-methyl-1-butanol, and phenylethyl alcohol. The individual concentrations of volatile compounds such as longer-chain ethyl esters, 3-methyl-1-butanol, and benzadehyde corresponded well to the intensities of related sensory attributes by the correlation analysis.

Funding support: Korea Food Research Institute

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Classification of Korean Black Raspberry Wines Using Consumer Preference Data with Fuzzy Reasoning and Cluster Analysis

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Fuzzy reasoning was applied to consumer preference data of 20 Korean black raspberry wines. A sensory attribute diagnostics was performed in terms of appearance, aroma, taste, and full body to determine overall acceptability of wines. First, the rating for the contribution level of each attribute to the overall acceptability was determined using ranking procedures. Then, the preference levels of each attribute and the overall acceptability were determined for 20 samples, using a 9-point hedonic scale by 96 consumers. The preference data were converted to fuzzy values and operated by fuzzy reasoning. Finally, the contribution and preference levels of each attribute were composed to infer the overall acceptability of the wines. The fuzzy preference ranking was the same as that of mean overall acceptability scores except two samples. Cluster analysis was performed to determine the clusters of samples based on the fuzzy distributions. Three clusters were determined and these three clusters also showed a good separation based on the mean overall acceptability scores of the samples. Discriminant analysis of fuzzy distribution based on three clusters also confirmed the same grouping of samples with 100% correctness.

Funding support: Korea Food Research Institute

Variation in Concentration and Composition of California Cabernet Sauvignon Tannins

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In order to develop an improved understanding of the extent to which tannin chemistry can vary within a single variety, samples from 38 blocks (three replicates) of Cabernet Sauvignon sourced from regions of California ranging from the southern San Joaquin Valley to Sonoma were studied. Blocks represented variation in allocation grade as assessed by the cooperating winery and growing climate. Tannin information was collected from exhaustive extracts of isolated skin and seed tissue as well as from a partial extraction system designed to investigate the potential impact of physiological integrity of tannin extraction. Extracted tannins were purified by solid-phase extraction and analyzed by acid-catalyzed cleavage in the presence of excess phloroglucinol, which provided information on subunit composition and average degree of polymerization. In addition, purified tannin extracts were analyzed by gel permeation

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chromatography (GPC), which provided information on tannin size distribution and flavan-3-ol amount. For berry samples, berry weight varied from 0.72 to 1.36 gm/berry and 1.3 to 1.8 seeds/berry with no observed geographical relationship. For exhaustive seed extracts, the tannin average molecular mass at 50%, as determined by GPC, varied from 2331 to 3646 molecular mass units, with concentrations ranging from 1.2 to 3.1 mg/gm berry weight for tannin and 0.07 to 0.36 mg/gm berry weight for flavan-3-ol monomers. For exhaustive skin extracts, (-)-epigallocatechin subunits comprised 34 to 48 mol% of total extension subunits. The proportion of skin tannin in the partial extraction system varied from 36 to 86%, suggesting a wide potential variation in wine skin tannin contribution. Overall, the results from this study indicate that variation within growing region exceeds that across regions representing a large climatic variation, thus suggesting that management practices can have a great impact on fruit tannin concentration and composition.

Funding support: American Vineyard Foundation

Comparison of GC-MS Aroma Chemical Profiles of Wine from Five Cultivars

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If the profiles of volatile aroma chemicals of wines from Rkatsiteli, Chambourcin, Traminette, Noiret, and Corot noir cultivars are analyzed on GC–MS after direct wine injection, then these profiles not only can be examined for similarities and differences among themselves for aroma volatiles but also may have different profiles than those analyzed by GC–MS after injection of volatiles extracted from the wines. Results of volatile aroma chemical profiling of wines from five different cultivars, some of which have not had aroma profiles published, by different sample preparations are examined for differences and similarities.

Funding support: Colorado Association for Viticulture and Enology and Colorado State Agricultural Experiment Station

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Retronasal Detection and Consumer Rejection Threshold of Brown Marmorated Stink Bug Taint in Commercial Pinot noir

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The Brown Marmorated Stink Bug (BMSB) is a crop nuisance that originated in Asia but has since spread across the United States. This insect has been found in grapes during the harvest period and subsequently has contaminated the crop through processing. BMSB taint occurs when defensive compounds are excreted by stink bugs disturbed during processing, specifically *trans*-2-decenal. The minimum levels of BMSB taint in wine at which consumers can detect its presence and reject the wine are currently unknown. Knowledge of these levels is crucial in determining the point at which control measures are warranted. In current work, the detection threshold and consumer rejection threshold of *trans*-2-decenal was measured in commercial Pinot noir. Triangle test with ascending forced choice method of limits and paired preference test with a method of constant stimuli were used to determine the detection threshold and consumer rejection thresholds, respectively. The results indicate that the consumer rejection threshold was much closer to the detection threshold than originally anticipated, suggesting that the detection of *trans*-2-decenal from BMSB can adversely affect wine quality. The spiked wine was described as cilantro, green which is consistent with previous work on this taint. These threshold levels are important to help wineries and other regulating bodies establish control levels for *trans*-2-decenal. Further work will determine threshold levels in other wine styles and the densities of BMSB in grapes at which threshold levels of *trans*-2-decenal are produced.

Funding support: Oregon State University

Changes in Anthocyanins throughout the Processing of Muscadine (*Vitis rotundifolia*) Wine

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Vitis rotundifolia species grapes (common name Muscadine) are rich sources of phenolic compounds, some of which are purported to provide health benefits. The anthocyanins, a subgroup of the phenolic and flavonoid groups, are responsible for the intense pigmentation in the grapes. In the current study, the anthocyanins in *Vitis rotundifolia* var. Ison were measured throughout the wine production process. Samples of a single crop of *V. rotundifolia* var. Ison

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grapes were collected at intervals throughout the vinification procedure, and the phenolics and anthocyanins were analyzed. In addition to the must and finished wine, the skins, juice, and press cake were also extracted and tested to assess the distribution of the polyphenolics and anthocyanins during the vinification process. Polymerized anthocyanins were analyzed through two methods: a mixed-mode C_{18} column was used for HPLC analysis and potassium metabisulfite bleaching was used for spectrometry. Cyanidin, malvidin, petunidin, peonidin, delphinidin, and pelargonidin dihexoses all rose in concentration in the wine must until the pressing step, when the skins were removed. After pressing, a sharp decline in anthocyanin concentrations in the wine must was observed, possibly due to the addition of refined sugar, due to sulfur dioxide present (<10 ppm). Certain anthocyanin forms were more affected by this drop than others. Total phenolic levels did not exhibit this same decline. Polymerized anthocyanins were not detected by either method. These data show that the anthocyanin forms within the Ison variety are resilient to the enological process.

Funding support: Louisiana State University Department of Food Science

Extraction of Proanthocyanidins during Fermentation of Muscat Bailey A Wine

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Muscat Bailey A (hybrid grape: Muscat Hamburg x Bailey, MBA) is native to Japan and its wine is very popular in Japan. MBA wines have extremely low concentrations of proanthocyanidins (PA) (65 mg/L) compared to wines made of other varieties, such as Cabernet Sauvignon(CS) (312 mg/L) and Merlot (356 mg/L). Proanthocyanidins were extracted from MBA seeds in model wines and their concentrations were compared to those of CS wines. For CS, 1500 mg/L of total phenols (TP) was extracted and final PA concentration was approximately 800 mg/L. For MBA, 300 mg/L of TP was extracted, but only a negligible amount of PA was detected. Wines were made with or without skins and seeds of those two cultivars. For CS, the highest PA concentration during vinification of normal red wine (with seeds and skins) was 536 mg/L, in which 183 mg/L (34%) was from seed and 379 mg/L (70%) was from skin. Seed PA concentration in CS wine increased continuously during the 13-day maceration period. In contrast, skin PA concentration peaked on day 9 of maceration and decreased thereafter. For MBA, the highest PA concentration detected was 96 mg/L, in which 26 mg/L (27%) was from seed and 59 mg/L (61%) was from skin. Skin PA concentration in MBA wine also decreased from day 6 of maceration. The data revealed that PA concentration in MBA wine was low because

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the amounts of extractable PA from both seed and skin were low. The final TP concentration was 2,107 mg/L for CS wine and 1,013 mg/L for MBA wine, and the difference was only two-fold. In contrast, the final PA concentration was 485 mg/L for CS wine and 19 mg/L for MBA wine, and the difference was 25-fold.

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Comparison of Freeze-Thaw and Cold-Soak Prefermentation Treatments on Phenolic Extraction in Syrah Wine Production

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Only a handful of controlled studies have examined the impact of freeze-thaw lysis on red wine production. We hypothesized that freeze-thawing Syrah grapes would lyse seed and skin cells more efficiently than simple cold soaking, allowing for greater phenolic extraction in finished red wine. Syrah grapes from two vineyard sites in San Luis Obispo County were harvested at two different levels of maturity and mechanically destemmed and crushed. Samples of control grapes were stored in 5 gallon buckets at 4°C, while freeze-thaw fruit was frozen in an industrial freezer at -26°C for 48 hours then thawed for 2 to 3 days. Winemaking procedures were identical for all samples. Triplicate fruit samples from each treatment were inoculated with a commercial yeast strain in 120 L stainless-steel fermenters for alcoholic fermentation. Wine was pressed, and the triplicate samples from each treatment were combined in stainless-steel kegs prior to ML inoculation. Phenolic content of postfermentation samples from each treatment are being quantified using a modified Harbertson–Adams assay, and HPLC is being used to analyze specific phenolic compounds, including catechin, caftaric acid, epicatechin, malvidin glucoside, monomeric/polymeric anthocyanins, resveratrol, and tannin. Postfermentation sensory difference tests with finished wine samples are in progress along with further sensory characterization of the finished wines. The results may indicate that freeze-thaw lysis can provide a reproducible technique to enhance phenolic extraction in red wine production.

Funding support: Cal Poly University, Wine and Viticulture Department

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Analysis of Heterocyclic Acetals in Wine as Markers of Age and Oxygen Exposure

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At low levels, oxygen can play a significant and positive role in the development of wine flavor and aroma. High oxygen exposure, however, can lead to undesirable changes. Consequently, methods for monitoring the degree to which wines have been exposed to oxygen are essential for controlling oxidative changes in wines. Nonenzymatic oxidation of wine results in the production of hydroxyl radicals, which are capable of reacting with most organic compounds in proportion to their concentration in wine. Oxidation of wine results in significant acetaldehyde production due to the large portion of the wine matrix constituted by ethanol. The interaction of acetaldehyde with glycerol, present at stable concentrations as high as 4 g/L, produces heterocyclic acetals. The high concentration of these reactants makes for reproducible and favorable kinetics in the production of the four stable isomers. Acetal isomers were extracted from new and aged Cabernet Sauvignons and analyzed by gas chromatography and mass spectrometry (GC–MS). The addition of hydrogen peroxide to young Cabernet was made to investigate the effect of induced oxidation on the production of these compounds. As it has already been demonstrated that the 5- and 6-membered ring structures of these acetal isomers are produced in a specific ratio found to change visibly over time in wine, this ratio is thereby a quantification of the extent of oxidation to which the wine has been exposed. Continued experimentation promises to validate the use of these compounds as markers of wine oxidation. Verifiable determination of wine age is still elusive. When combined with only minor additional chemical details of the life of the wine, this process of quantifying the isomeric ratio of heterocyclic acetals may prove to be a rapid and accurate diagnostic for the determination of wine age.

Funding support: University of California, Davis

Metabolic Basis of Sluggish Fermentations in Difficult to Ferment Juice

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Selection of yeast suitable for the specific juice composition and fermentation conditions of the winery and maintenance of permissive growth conditions will generally reduce the incidence of fermentation arrest. However, there are juices that chronically display sluggish fermentations that are not corrected by

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nutrient supplementation or yeast strain selection. These "difficult to ferment" juices typically show a common profile of low YAN, relatively high proline, and low arginine, and the fermentation curves display an early transition to a reduced fermentation rate but are not rescued by amending the YAN deficiency. Three different commercial yeast strains with differing nutritional requirements, UV43, D254, and L2226, were used to assess fermentations in three different juices, one from a Chardonnay vineyard producing historically difficult to ferment juice and two other Chardonnay juices from different regions that typically ferment without incident. All the three strains took the longest, 13 to 18 days, to finish the fermentation in the difficult to ferment juice compared to 8 to 12 days in the other Chardonnay juices. The difficult to ferment juice had lower YAN, but still within the fermentable range. Metabolomic analysis was performed on these fermentations to identify the differences in intermediary metabolites that may give an insight to the factors leading to these types of sluggish fermentations. Six replicates of each fermentation were run and samples collected on day 4 for metabolomic analysis. At day 4, all three difficult to ferment Chardonnay lots had slowed compared to normal progression for the other two juices. Preliminary results from PCA analysis of the complete metabolite profiles shows the three juices are consistently grouped separately. The metabolic profiles from the difficult to ferment juice showed some common features of reduced levels of compounds associated with growth and higher levels of compounds associated with membrane components. Strain-specific and juice-specific patterns were also observed.

Funding support: American Vineyard Foundation

A Rapid Approach to Measure Grape Fractions Reactivity toward Saliva by Means of the Saliva Precipitation Index

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Saliva may represent an interesting tool by which astringency of red wine can be evaluated. The perception of this tactile sensation derives from the formation of a protein-tannin complex which precipitates and causes the shrinking, drawing, and puckering of the epithelium. Wine tannins derive from the diffusion and extraction of phenolics from grape skins and seeds during winemaking. The reactivity of polyphenolic fractions of Aglianico, Merlot, and Cabernet Sauvignon grape (*Vitis vinifera*) skins and seeds toward salivary proteins was evaluated by means of an analytical method based on the SDS-PAGE of human saliva. The method has the aim to simulate the physiological conditions during tasting and furnish an index (saliva precipitation index, SPI) to objectively evaluate astringency. In this work a rapid and automated electrophoresis was applied to obtain the SPI of grape tannins and results were

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compared to that obtained by the classical electrophoresis. The optimized method resulted in an improved efficiency as the reduction of time working reached ~67%. Since the SPI was found to be well correlated with the sensory analysis, a measure of the astringency of grape skin and seed tannins dissolved in hydroalcoholic solution at a concentration of 1 g/L was carried out. In addition, a characterization of phenolics by HPLC-MS analysis was performed in order to correlate the proanthocyanidin characteristics with the precipitation of salivary proteins.

Funding support: University of Naples and Laffort Enologie

Consumer Rejection and Sensory Threshold of 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) by Wine Consumers

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Important to the aroma profile of a mature Riesling is a "petrol" or "kerosene" note. This note is due to the presence of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). Estimates of TDN thresholds in wine have been reported, but the TDN concentration that affects consumer acceptability is not clear. To address this question, we combined two methods: threshold determination and paired preference to determine the consumer rejection threshold. Both of these studies were conducted in replicate at two locations, New Zealand (Lincoln) and the United States (Pullman). The two populations allowed us to determine differences in consumer rejection thresholds of TDN in Riesling across regions. Regular white wine consumers (n = 36) performed threshold testing using the forced-choice ascending concentration series method, with TDN concentrations of 6.25, 20, 64, and 204.8 µg/L. In addition, consumers received pairs of Riesling samples, one spiked with each concentration of TDN and one control, and were asked to indicate which sample was preferred. Results showed that the TDN threshold value in Riesling for the New Zealand and U.S. consumers was 37.9 and 43.2 µg TDN/L, respectively. Over duplicate panels in both locations, no significant differences were observed in threshold values ($p > 0.05$). However, differences in TDN preference between the two regions were observed. In the United States, the consumer rejection threshold was 64 µg TDN/L, while in New Zealand, the consumer rejection threshold was 204.8 µg TDN/L. These results suggest that consumers in both regions detected the presence of TDN at similar concentrations but that preference or tolerance of TDN in Riesling varied, with the New Zealand consumers tolerating a higher concentration of TDN. These results highlighted the influence of TDN on acceptance of Riesling and may be useful for those winemakers and marketers wishing to address differences in consumer preferences across regions.

Funding support: Washington State University, Lincoln University

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Red Wine Quality Assessment Using the Electronic Tongue

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Continuous improvement of wine quality requires the use of novel research approaches to rapidly and objectively assess wine quality. The objective of this study was to use the Astree electronic tongue to distinguish among wine samples and correlate these instrumental measures with sensory data. Five wine treatments were analyzed for sweetness, bitterness, metallic, spiciness, and sourness using the Astree electronic tongue: control (28 Brix), water back to 19 Brix, water back to 19 Brix + sugar to 28 Brix, water back to 23 Brix, and water back to 23 Brix + sugar to 28 Brix. Trained panel sensory evaluation for sourness, astringency, and bitterness were also included in model development to determine the strength of agreement between the electronic tongue and sensory data. Principal component analysis was used to visualize sample separation and the relationship of the response of the electronic tongue sensors to the sensory attributes. The results showed a high discrimination index (DI > 80) among wine treatments using the electronic tongue. Principal component (PC) analysis (PC1 and PC2) explained a large amount of the variation (97%) observed among the wine treatments. More specifically, the control sample was associated with sweetness, bitterness and umami notes. While water back to 19 Brix and water back 19 Brix + sugar were predominantly associated with sourness, the remaining two samples (water back to 23 Brix and water back to 23 Brix + sugar) were separated based on higher metallic notes. Over all the samples, a high positive correlation ($r > 0.90$) was observed among ethanol content, perceived astringency and bitterness intensities, and the electronic tongue data. Considering the strong relationship between the electronic tongue data and the trained sensory evaluation data, the Astree electronic tongue shows promise as an innovative approach for the rapid and objective evaluation of wine quality.

Funding support: Washington State Grape and Wine Research Commission

Convenient Measurement of Free SO_2 by Gas Detection Tubes

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Aeration-oxidation (A-O) is widely used for quantifying free sulfur dioxide (SO_2) in wines. We describe a modification of A-O that requires less expensive equipment and less time than conventional analyses and that may be preferable in modestly equipped wineries. In the modified method, an acidified wine sample was treated with an antacid tablet to evolve CO_2 in situ and the gas flow directed to a commercial SO_2 gas detection tube which darkens proportion-

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ally to SO_2 exposure. The gas tube method was initially calibrated against A-O using model wines containing 5 to 40 mg/L SO_2. The method accuracy was then evaluated by measuring free SO_2 in commercial wines (n = 16) using both the gas tube and A-O methods, and measurements between the two methods were within ±2 mg/mL for all wines. The limit of detection of the SO_2 tube method was slightly worse than A-O (3.3 mg/L in wine), and the major source of imprecision appeared to be determining the start and stop of darkening.

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Inline Multispectral Colorimeter for Real-Time Color and Total Phenolic Analysis during Red Wine Fermentations

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A phenolic sensor was developed for inline determination of color and total phenolics during red wine fermentations. The colorimeter uses multiple light emitting diodes (LEDs) spanning the ultraviolet and visible spectrum. The performance of the colorimeter was tested on red wine fermentations during the fall 2012 season at UC Davis Robert Mondavi Institute. Inline measurements were performed during the complete fermentation period of two Cabernet Sauvignon and one Pinot noir wines. Manual samples were also taken during the fermentation and benchmarked against two instruments. These samples were immediately refrigerated and subsequent to fermentation were centrifuged and measured. One measurement was with a commercial UV-Vis spectrometer and the other with the LED colorimeter. In an independent experimental study, manual samples were taken from a diverse set of nine fermentations and characterized by the UV-Vis spectrometer and the LED colorimeter. The studies show that the inline LED colorimeter can measure the color and total phenolics during fermentation. A strong correlation exists between inline LED measurement and the off-line UV-Vis colorimetric measurements. The study demonstrated the validity of the inline colorimetric approach in the presence of wine samples with suspended particles.

Funding support: Rodgers University Fellowship in Electrical and Computer Engineering

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Enology – CONTINUED

Influence of Viticultural and Prefermentative Measures on Pyrazine Level and Sensory Properties of Sauvignon blanc

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The aroma of Sauvignon blanc is mainly influenced by two groups of compounds. Pyrazines are responsible for the greenish character, while thiols influence the fruitiness of the wine. Both are highly aroma active at low ng/L levels. The balance between formation, preservation, and degradation of these substances are directly linked to the sensory profile and aroma composition of the wine and can be influenced in the vineyard as well as during the early stages of grape processing prior to fermentation. Among the viticultural influences, vine vigor due to modified nitrogen disposability and leaf removal was examined. The influences of skin-contact time and degree of clarification were also selected as examples for the enological measures. Chemical analysis was performed by FT–MIR spectroscopy and enzymatic assays. Aroma compounds were analyzed with stable isotope dilution assay by MDGCMS after solid-phase extraction. Sensory profiling and preference tests were performed six months after bottling. The results show that cold soak significantly increases the extraction of polyphenols during the first 24 hours as well as the level of methoxypyrazines with no direct correlation between these two parameters. Increasing concentrations of solids on the other hand do not influence the polyphenol level but lead to lower final concentrations of methoxypyrazines. Surprisingly, preference tests revealed that wines without skin contact and very low concentration of solids are preferred. In a descriptive sensory analysis, these wines were described with fruity characters and lack the green and reductive attributes. Highly water- and nitrogen-stressed vines led to untypical, fumy tainted wines, while unstressed vines with high vigor and crop load were judged to lack in fruity characters such as gooseberry or passion fruit. In summary, both well-balanced wine vigor and precise must clarification result in fruity typical Sauvignon blanc wines.

Funding support: Competence Center for Wine Research

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Development of a Microvinification Procedure to Predict Problematic Malolactic Fermentation of Cool-Climate Chardonnay

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Ensuring the completion of malolactic fermentation (MLF) in a timely fashion is important for maintaining the integrity of wines and for smooth running of the cellar. Currently there is no way of predicting stuck or sluggish MLF before it arises. We investigated the ability of the rate of depletion of malic acid during MLF of Chardonnay in a flask to predict the extent of completeness of MLF in a tank on a commercial scale. Samples of 11 lots of cool-climate Chardonnay must were taken after yeast inoculation in commercial-scale tanks and alcoholic fermentation was conducted in small volumes on a laboratory bench-top. At dryness each wine was divided into triplicate 250 mL aliquots and inoculated with commercial strains of *Oenococcus oeni*. Depletion of L-malic acid was monitored with enzymatic assays from the day of inoculation until full depletion or the end of the research period. Depletion of malic acid in corresponding tanks was monitored weekly after inoculation. Depletion of malic acid in flask was compared with that in tank through visual comparison of depletion curves and simple linear regression. The relationship between duration of lag in flask and lag in tank was statistically weak, but duration of lag in flask was a stronger indicator of duration of lag in tank than any single chemical parameter measured. Both methods of analysis show that results differed somewhat by strain of malolactic bacteria. Of the seven lots with <8 days lag in flask, five had <90 days of lag in tank. Of the three lots with >8 days lag in flask, two had >90 days of lag in tank. With some refinement and repeated testing, the procedure developed in this research might prove to be a useful method for commercial winemakers to predict problematic malolactic fermentation of cool-climate Chardonnay.

Funding support: Edna Valley Vineyard

Identifying Consumer Attitudes that Impact Willingness-to-Pay for a Nutraceutical-Rich Juice Blend

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Determining the impact of consumer attitudes on the valuation of a product is a critical phase of product development and marketing. Consumers may have differing attitudes that influence their willingness-to-pay (WTP). A non-hypothetical auction-like mechanism was utilized to identify consumer attitudes that impacted WTP for a 946 mL bottle of a nutraceutical-rich juice blend

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(75% Concord+12% Pomegranate+13% Black Cherry). Participants (n = 228) were divided into four groups (Info, Taste, InfoTaste, and Control) to determine WTP. The Info group received a potential health statement, the Taste group evaluated the sensory attributes of the juice blend, the InfoTaste group evaluated the sensory attributes and received the health statement, and the Control group neither tasted the juice nor received the health statement. Participants also completed a questionnaire to evaluate their agreement to health-related statements and measure their time preference (future orientation). The average WTP for the juice blend was $3.45/bottle. WTP varied by treatment group ($3.65/bottle for the Info group, $3.51/bottle for the Taste group, $3.28/bottle for the InfoTaste group, and $3.39/bottle for the Control group). In terms of health-related statements, consumers strongly agreed that antioxidants were beneficial to health (64%), they preferred products that claimed to be 100% juice (72%), they preferred products that were pure (52%), and they were willing to pay more for expensive fruit juices that were healthier (59%). WTP was positively correlated to agreement that antioxidants were beneficial (Info), agreement that high fructose corn syrup is less desirable than sugar (InfoTaste), and WTP for more expensive, healthier fruit juices (Control). Individuals with less future orientation may not be as protective of their health as those with more future orientation and thus were willing to pay less for health-protective products. Experiments using non-hypothetical mechanisms offer realistic insight into consumer attitudes to aid in product development and marketing.

Funding support: USDA Specialty Crop Research Initiative

Effects of [*GAR*$^+$] Prion State on the Physiology and Membrane Composition of *Saccharomyces cerevisiae* Wine Strains

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We have previously reported on the dominant heritable [*GAR*$^+$] prion in *Saccharomyces cerevisiae* wine strains. This yeast prion acts like a stress-induced switch, allowing cells to bypass an otherwise extreme preference for hexoses. [*GAR*$^+$] cells can utilize alternative carbon sources but at the cost of reduced glucose and fructose consumption. Cells that induce [*GAR*$^+$] experience a massive drop in *HXT3* expression, a hexose transporter that plays an important role in fermentation. However, loss of *HXT3* alone is not sufficient to explain all the phenotypes of [*GAR*$^+$], so we began characterizing physiological differences between wild-type prion minus [*gar*$^-$] cells and prion positive [*GAR*$^+$] cells. Other than the ability to bypass glucose-associated repression and its effect on fermentation kinetics, [*GAR*$^+$] appears to confer a variety of physiological effects that impact fundamental cellular processes. In general

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cells that harbor [*GAR*$^+$] appear to grow slower than [*gar*$^-$] cells. They are more sensitive to temperature and ethanol, but can remain viable for significantly longer than [*gar*$^-$] cells. Cellular morphology of [*GAR*$^+$] cells is highly variable and can resemble pseudohyphal growth. This difference in morphology and growth rate is reflected in the lipid profile of [*GAR*$^+$]. After 24 hours in synthetic juice, [*GAR*$^+$] cells had elevated levels of phosphatidylinositol (PI) relative to [*gar*$^-$] cells at the same time point. This high ratio of PI is indicative of nutrient depletion and resembles stationary phase cells. However, [*GAR*$^+$] strains do not display a classic stress response and are more sensitive to extremes of temperature and ethanol. Our hypothesis is that [*GAR*$^+$] cells are in a novel stress-induced "bet-hedging" state that dynamically affects fundamental cellular processes like nutrient uptake, metabolism, and homeostasis. Our goal is to develop a set of biomarkers to identify [*GAR*$^+$] cells and ultimately help winemakers diagnose and resolve sluggish and arrested fermentations.

Funding support: Paul Monk Scholarship, Wine Spectator Scholarship, American Wine Society Education Foundation

Molecular Markers of Microoxygenation of Red Wines

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The direct treatment of wine with low amounts of oxygen known as microoxygenation (MOX) has become a standard practice for improving the body, structure, and fruitfulness of red wines. Chemical changes known to occur during MOX include aldehyde-cross linkage of polymeric tannins and the production of stable pigments. How oxidation changes flavor and aroma, however, is still very much an open question. An experimental apparatus was developed to allow for the controlled introduction of oxygen into laboratory-scale volumes of wine at typical winery MOX rates. Oxygen was introduced to the wine through gas-permeable tubing. The wine was monitored for changes in oxygen concentration, acid concentrations, phenolics, carbonyl-containing compounds, volatile components, and off-gassing. Three varieties of red wine were microoxygenated at a rate of 30mg/L/month for up to five weeks to find markers of oxidation. Tentatively identified as possible markers included the following: ethyl esters of long-chain fatty acids (C8 to C12), 2-octanone, diethyl succinate, coutaric and caffeic acids, procyanidin C, and several unidentified phenolic compounds. This experiment also showed that MOX and/or slow stirring of wine promoted the production of gas from the finished wine in an episodic manner. Dissolved oxygen levels in the wine varied with the wine variety, but was generally very low and remained very low throughout the treatment period. The results of this experiment suggest that MOX of red wine

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may induce changes that are mediated by microbial metabolism to a degree dependent on the concentration of viable microbes present in the wine. Such metabolism would prevent direct chemical oxidation of wine components. Thus, MOX of wine with microbes present may be mediated by the microbes instead of by direct chemical reactions of the wine with oxygen.

Funding support: E&J Gallo

Impact of Prefermentation Cold Maceration and Yeast Strains on Volatile Composition of Gewürztraminer Wine

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Gewürztraminer is a well-known aromatic grape variety that produces full-bodied white wines with excellent flavor characterized as Traminer, tropical fruit, and lychee-like aroma. Phenylethanol, phenethyl acetate, *cis*-rose oxide, and wine lactone have been reported to be the most important contributors to the aroma of Gewürztraminer wine. The objective of this study was to investigate the impact of prefermentation cold maceration and yeast strains on the aroma of Oregon Gewürztraminer wine. The grapes were treated with high hydrostatic pressure (HHP) to inactivate naturally occurring yeast and bacteria before inoculation. Fermentations were conducted at the Oregon State University research winery. Prefermentation cold maceration was conducted for 7 days at 8°C. Following cold maceration, all ferments were warmed to 13°C to complete the alcoholic fermentation with a commercial yeast (*Saccharomyces cerevisiae* VIN13) and a yeast isolated previously at OSU with high glycosidase activity. No cold maceration treatment was conducted directly at 13°C with yeast inoculation and alcoholic fermentation. Gas chromatography/olfactometry (GC/O) and solid-phase microextraction (SPME)-GC-MS methods were applied to the analysis of flavor volatiles of Gewürztraminer wines. Quantitative results showed that the yeasts had significant impact on the aroma profile of the wines. The yeasts generated different profiles for main fermentation esters, alcohols, and some volatile phenolic compounds; however, the two yeasts generated similar concentration of linalool, citronellol, *cis*-rose oxide, and β-damascenone.

Funding support: Oregon Wine Board

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Behavior and Activity of Selected Yeast Species in Hydrolyzing Pinot noir Glycosides

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Terpene alcohols and C_{13}-norisoprenoids are present in grape as free form and as glycoside conjugates. Glycoside conjugates are nonvolatile and flavorless, but they could be hydrolyzed by glycosidic enzymes during fermentation and enhance wine quality. Previous studies have shown that some *Saccharomyces cerevisiae* and non-*Saccharomyces cerevisiae* yeast strains had high glycosidic enzyme activity. The objective of this study is to investigate the behavior and ability of selected yeast species in hydrolyzing glycosides isolated from grape. Grape glycosides were isolated from Pinot noir grapes using C18 reversed-phase solid-phase extraction cartridges. Isolated glycosides were kept in freezer in methanol solution and dried right before use. Seventeen selected yeast strains including 10 non-*Saccharomyces cerevisiae*, five *Saccharomyces cerevisiae*, and two unknown were tested on the glycosidase activity in synthetic medium, at 8°C and 25°C. The yeast strains were first streaked from slants onto YPD plates and single colony was inoculated in MYGP medium to reach 10^7 cfu/mL. The harvested yeasts were then inoculated in sterile-filtered YNB medium with dry isolated glycosides, for 48 hours at 8°C and 25°C. Released flavor compounds were analyzed by SPME-GC-MS. Results demonstrated that most of the selected yeast strains can liberate both terpene alcohols and C_{13}-norisoprenoids in the synthetic medium at both temperatures and higher glycosidase activities were observed at higher temperature for most of the yeast strains. In general, the selected *S. cerevisiae* yeasts showed higher activity in the selected medium than most non-*Saccharomyces cerevisiae*. Two of the non-*Saccharomyces cerevisiae* yeast strains (*Kluveromyces thermotolerans* and *Metschnikowia pulcherrima*) showed activities similar to the selected *S. cerevisiae*.

Funding support: Oregon Wine Board

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Survey for Grapevine Red Blotch-Associated Virus in the Foundation Plant Services Vineyards

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In 2008, a new disease characterized by red blotches along leaf margins and red veins under the leaf surface was observed in red grape varieties in a few vineyards in Napa Valley, California. A new DNA virus, Grapevine Red Blotch-associated virus (GRBaV) was identified in these vines using next generations (NGS). GRBaV has subsequently been identified in Cabernet franc, Cabernet Sauvignon, and Zinfandel vines in California, New York, Pennsylvania, and Canada. Given the apparent widespread incidence of GRBaV and the unknown nature of its origin and mode of transmission, we decided to screen a majority of the planting stock at Foundation Plant Services for GRBaV. This planting stock included all the vines in our newly established Russell Ranch vineyard in addition to more than 1,600 vines from our Classic Foundation vineyard. To facilitate screening a large number of vines, we developed a new real-time PCR assay using primers from a conserved region of the GBRaV genome. Comparison of the real-time PCR test results with those from conventional PCR for the 1,102 Russell Ranch vines verified that our new assay was highly sensitive and specific for GRBaV and indicated that all Russell Ranch vines were negative for GRBaV. More than 1,600 vines from the classic Foundation Vineyard are also being tested for the virus. The infection rate in the Classic Foundation was less than 0.2% (3/1600). Fourteen samples in close proximity to the three GRBaV-positive vines were also collected and tested. Of these, six were sibling vines. These vines were all negative for GRBaV. As a leading source of orchard and vineyard planting stock in the United States, in addition to being a central component of the Grape Clean Plant Network, these negative results indicate that FPS stock is not a major source of GRBaV spread within vineyards.

Funding support: Foundation Plant Services

Tracking *Vitis vinifera* cv. Cabernet Sauvignon Phenolics throughout Ripening: A Two-Year Study Utilizing an Isotopic Tracer

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Understanding the regulation of phenolic compounds in agricultural products has been a topic of great interest. In *Vitis vinifera* berries, phenolics are responsible for important sensory and functional characteristics. To shed light on the

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ripening profile of phenolic compounds in Cabernet Sauvignon berries, the stable-isotope tracer L-phenyl-$^{13}C_6$-alanine (Phe13) was incorporated in situ and the development of labeled and unlabeled phenolics was tracked in the vineyard at different stages of maturity over two vintages. Phenolic profiles during ripening were consistent with previous research; however, individual anthocyanins accumulated with different profiles during ripening: malvidin species continually climbed in concentration while other anthocyanins tended to plateau or drop near the end of the growing season. The isotopic label was predominantly incorporated into anthocyanins, presumably because of their dominant accumulation during ripening. Notably, the incorporation of label continued long after levels of Phe13 had dropped to below 1 nmol/berry, preventing an accurate assessment of the hypothesized turnover of anthocyanins. While our tracer did not perform exactly as we had expected, the results of this study suggest the presence of a previously unreported pool of phenolic pathway substrate.

Funding support: American Vineyard Foundation

Analysis of Subjectivities about Leafroll Disease Management among Napa Grapegrowers and Winemakers

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A Q-method approach was used to assess the opinions concerning *Grapevine leafroll-associated virus 3* (GLRaV-3) management. Three workshops were held in the Napa Valley at which invited participants were asked to write down their views in response to a set of open-ended questions about leafroll, its impacts, and the prospects for cooperative management of the disease. Responses were sorted into thematic groups (e.g., statements about financial issues, clean plant material, interpersonal trust). A small subset of response statements which encapsulated the groups of opinions was extracted. This resulted in a set of 47 statements. Invitations were issued via email and by personal contacts to a further group of participants drawn from the Napa Valley grower and winemaker communities, generating a participant group of 37 individuals. These people were interviewed on a one-to-one basis and the participants ranked the statements by the degree to which each one accorded with their own views. The interviews were conducted during the fall and winter months of 2011. The resulting two-way table of data, in which each row gave the numerical rank assigned to each statement by one participant, was then subjected to a principal component analysis to extract information regarding the distribution of opinions over the group of participants and to identify meaningful classifications of the responses. The analysis revealed a wide diversity of opinion distributed among some broad categories of response.

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Impact of Irrigation Strategy on Vine Physiology, Nutrients, Yield, and Fruit Quality of Tempranillo in Southern Oregon

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Until recently, there has been no formal effort to develop and implement sustainable guidelines for irrigation practices in southern Oregon. The objective of this study was to determine the effects of sustained and regulated water deficit on vine physiology and berry composition of Tempranillo grapes located in two different American Viticultural Areas in southern Oregon (Rogue Valley and Umpqua Valley). Each site was characterized by different type of soil. The following treatments were imposed when leaf water potential reached -1.2 MPa: (SD-1) initiate irrigation at 70% of ET_c until harvest (control-standard industry practice); (SD-2) initiate irrigation at 35% of ET_c until harvest; (RDI-1) initiate irrigation at 70% ET_c until veraison, then 35% of ET_c to harvest; and (RDI-2) initiate irrigation at 35% of ET_c until veraison, then 70% of ET_c until harvest. Midday leaf water potential (ψ_{md}) decreased significantly by the end of the season in all irrigated treatments. RDI-1 had consistently lower values compared to the other irrigation treatments. Significant variation in the nutrient status was found not only among the irrigation treatments but also from vintage to vintage. The irrigation treatments had a significant impact on potassium and nitrogen and varied significantly from site to site. Brix, pH, and anthocyanins were affected by the irrigation strategy applied. The magnitude of variation was highest in deep soils. The yield components were affected by the irrigation strategy at both sites. Yield was affected by the irrigation treatments mainly because of the number of clusters, which overall was higher in the treatments from the deep soil than in those from the shallow site. Tempranillo indicated to be very sensitive at water status variation.

Funding support: Oregon Wine Board

A First Year Evaluation of the Springtime Temperature Inversion Conditions throughout Three California Counties

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There is increasing interest in the use of wind machines for frost protection as water supplies for sprinkler frost protection become more limited. The ability of wind machines to provide useful levels of frost protection depends upon the strength and reliability of the temperature inversion conditions during frost events. However, little information currently exists to describe inversion conditions on a regional basis in California, which creates uncertainty in choosing frost protection strategies. To address this shortcoming, we measured springtime

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temperature inversion conditions at 65 locations throughout vineyard regions of Sonoma, San Luis Obispo, and Santa Barbara Counties in 2012, as the first year of a planned three-year assessment. At each location, a 10.7 m high tower was installed in early to mid-March 2012 and remained in place until June 2012. Each tower had temperature data loggers at 1.5 m and 10.7 m heights, programmed to record air temperatures at 5-minute intervals. On average throughout the three counties, when the 1.5 m nightly minimum temperature fell to within the range of 0 to -1°C, useful temperature inversions (defined as greater than 1°C) were observed on 71% of the nights with an average strength of 1.90°C (the difference in temperature from 1.5 to 10.7 m). When the 1.5 m nightly minimum temperature was within the range of -1 to -2°C, useful inversions were observed on 75% of the nights with an average strength of 1.91°C. When the 1.5 m nightly minimum temperature was below -2°C, useful inversions were observed on 74% of the nights with an average strength of 1.95°C. The variability of the inversion patterns among individual towers was large; this indicates the strong influence that local conditions have on the formation of temperature inversions in these regions of characteristically hilly terrain.

Funding support: CDFA Specialty Crops Block Grant Program, American Vineyard Foundation

Comparison of Training Strategies and Vine Shelter on the Development and Productivity of Chardonnay Grapevines

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Training method and vine shelters were evaluated for their influence on growth and productivity during the first seven years of establishment of bilateral cordon-trained Chardonnay grapevines in the Salinas Valley. The experimental design was a split-split plot. Main plots were shelter type using a paper carton or a plastic growth tube. Subplots were all shoots allowed to grow or thinned to a single shoot the first year. Sub-subplots were vines pruned to a two-bud spur or a trunk developed at dormancy before the second year. Plastic shelters were shown to significantly increase the first year shoot length and diameter as compared to a paper carton. There were no benefits in the second and third season in increasing growth or production with the use of a tube. Thinning to one shoot per vine and using a tube did produce the longest shoots in the first year. Plastic tubes generally resulted in one shoot becoming dominant even when all shoots were left to grow. Thinning to one shoot per vine as measured at the end of the first year did not reduce total leaf area. The increase in the development of lateral shoots on the thinned vines compensated for the reduced shoots left on the plants. First year training methods had no effect on growth or production in the second or third season. The second year training method was the factor that had significant effects on vine growth and production in

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the second and third year. The practice of leaving a trunk resulted in more advanced development of the bilateral cordon vines when compared to vines that had the more traditional practice of pruning vines back to a two-bud spur. The more developed vines of the trunk treatment had a higher capacity to produce and ripen a crop in the third season.

Funding support: Monterey County Vintners and Growers Association and Valley Farm Management

Testing *Vitis arizonica* Candidate Genes for Pierce's Disease Resistance in *Nicotiana tabacum* SR-1

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The xylem-limited bacteria *Xylella fastidiosa* is the causal agent of Pierce's disease (PD) in grapevines and numerous other economically important crops. In an attempt to find a long-term sustainable solution to PD, the Walker lab has made significant progress breeding PD-resistant grapevines. The goal has been to obtain grapevines with resistance to PD from native American *Vitis* species while conserving the quality of elite *Vitis vinifera* wine and table grapes. Another long-term and sustainable approach is to genetically transform susceptible *V. vinifera* varieties with resistance genes cloned from PD-resistant North American *Vitis* species. This approach may have a more limited impact on the *vinifera* parent's fruit characteristics. The genetic mapping of a form of *V. arizonica* resulted in the localization of a homozygous dominant locus, designated *PdR1*, responsible for PD resistance. Physical mapping and sequence analysis around this locus resulted in the identification of five candidate genes. In order to fully characterize the function of these gene candidates, we are conducting complementation assays by transforming these *PdR1* candidates into susceptible *Vitis* cultivars and tobacco via *Agrobacterium tumefaciens.* At present, we have in vitro plants of Chardonnay, Thompson Seedless, and St. George as well as plants of *Nicotiana tabacum* SR-1 transformed with all five candidate genes. Tobacco plants with all five genes are currently being screened in a greenhouse. Transformed plants were inoculated twice between the second and third node and will be sampled four times at four-week intervals. Symptoms will be scored on a 5-point scale, and the samples analyzed with enzyme-linked immunosorbent assay. The response of the transformed tobacco plants to *X. fastidiosa* infection will be presented.

Funding support: CDFA Pierce's Disease/Glassy-winged Sharpshooter Board and Louis P. Martini Endowed Chair for Viticulture

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Feasibility of Growing Pierce's Disease Resistant 87.5% *Vitis vinifera* Grapes 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 (CREC) near Clanton, Alabama. The experimental vineyard utilizes a RCBD with six blocks and five 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 preliminary results suggest selection 502-10 had the lowest pruning weight of 0.75 kg in 2012, while 501-12 had the highest (1.2 kg). Selection 502-10 started to mature early in the season, while 501-12 initiated the veraison in mid-August and ripened late in September. The three selections differed in total yield per vine. The late maturing 501-12 produced the largest crop of 5.8 kg/vine. Bird feeding caused a considerable crop loss for the early ripening 502-10. Selection 501-12 produced the highest number of clusters per vine (76.8), while 502-10 had 12.5 clusters. Midseason selection 502-01 had the largest cluster weight of 173 g and produced the largest berries, while the late season selection 501-12 had the sweetest berries in 2012. Preliminary results for the newly developed PD resistant *V. vinifera* selections in Alabama are very encouraging. Knowledge gained through this project will aid in development of best management practices and production system recommendations, vital for the establishment of a sustainable grape industry, and enhance the competitiveness of Alabama-grown specialty crops.

Funding support: NIFA-USDA-ADAI-SCBP

Genetics of Plant Defense Responses to Pesticides on Grapes

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Pesticides are known to produce effects that go beyond their intended purpose. For example, on grape (*Vitis vinifera*), Pacific spider mite (*Tetranychus pacificus*) density increases several weeks after cessation of early- to midseason sulfur applications for control of powdery mildew (*Erysiphe necator*). A possible

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explanation is that pesticides interfere with genetic transcription factors of the plant, leading to alterations in production of plant defense proteins and phytoalexins (such as phenolics). We conducted laboratory and field tests to evaluate vine response to pesticides and spider mites. In the lab study, Chardonnay vines treated with sulfur dust or inoculated with *T. pacificus* were compared to an untreated control. In the field study, Cabernet Sauvignon and Chardonnay vines were treated with one of four pesticides, sulfur, trifloxystrobin, quinoxyfen, myclobutanil, or treated with myclobutanil and subjected to deficit irrigation. Vine genetic response was evaluated by extracting RNA, then reverse transcribing to synthesize a cDNA (complementary strand of DNA), followed by double-strand DNA synthesis, in vitro transcription of biotin-labeled aRNA (amplified RNA), fragmentation (into smaller base-pairs), and hybridization onto an Affymetrix *Vitis vinifera* microarray. The degree of gene expression was quantified and compared among treatments.

Funding support: UC/CSU Collaboration, CSU Agricultural Research Initiative

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

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Currently, rootstock decisions are primarily based on pest resistance factors and only partially on 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 the ripening period and the initiation of dormancy of a scion. The main objective of this research is to determine the role grapevine rootstock parentage has in scion phenology; specifically, alterations to the onset of senescence. This study is examining 101-14 Mgt and 110R planted in two separate rootstock trials. Significant differences between these rootstocks were found in scion internode length, shoot caliper above the second cluster, leaf-holding capacity over the course of the season, and canopy density via light bar measures. These physiological markers are a means of evaluating and classifying rootstock influence on scion growth, development, and senescence. Determining the role parentage plays in rootstock-scion interactions helps to guide rootstock breeding efforts. It would also allow growers to choose rootstocks that prolong leaf function and provide more time for sugar accumulation or to hasten maturity to better match climates with shorter, colder seasons, and hasten maturity to reduce vineyard water needs.

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

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Characterization of the Flavor Profile of Norton (*Vitis aestivalis*) Grapes at Different Stages of Maturation

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Norton is an interspecific hybrid in its parentage and is an economically important winegrape cultivar for the Midwest and Eastern grape industry. Norton's disease resistance and lack of "foxy" aroma make this an interesting cultivar for breeding new cultivars. However, Norton berries tend to retain high levels of malic acid, titratable acids, and potassium and exhibit an alkaline pH. Despite Norton's current economic importance, limited research has been conducted to characterize berry flavor and color development. In 2012, a study was initiated using established six-year-old grapes from Mountain Grove, Missouri. Samples were collected at seven different stages of maturation to track development of sugar and acid concentrations, as well as phenolic compounds. The accumulation of soluble solids (Brix) was linear through berry maturation and peaked at 106 days after flowering (DAF). At the final harvest date, 120 DAF, no change in Brix was measured; soluble solid values were 22.75 ± 1.14 and 22.32 ± 1.19 for 106 and 120 DAF, respectively. Changes in titratable acids, total sugars, and anthocyanins (flavonoid for color development) also were measured through berry maturation.

Funding support: Division of Plant Sciences, Caraker Law Firm Enology & Viticulture (scholarship for N.E.D.)

Prediction and Analysis of Berry Weight during Ripening Stage According to the Effects of Meteorological Conditions

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The objectives of this work were to develop and analyze a grape crop yield model to predict rates of ripening and harvest dates for grape cultivars throughout La Rioja (Spain). Data was gathered from Tempranillo berries in the Rioja Alta within the Designation of Origin Rioja in 14 locations for six years (2003–2008). Berries were sampled during the ripening stage (from 6 to 8 weeks) and meteorological data were gathered from 44 stations located in the nearby data sites. Based on the data collected from these stations, kriging methods are used to calculate the value of the meteorological variables in the analyzed plots, and models were generated and subjected to PCA transformation. Once the input variables were prepared, an algorithm based on Gaussian Processes was used with a Radial Basis Function kernel to obtain a model with a RMSE of 9.39%. Subsequently, weight

*indicates corresponding author

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behavior was simulated to determine the influence of atmospheric conditions with a variation of these variables according to historical values for Rioja. Finally, a decision support tool was developed to help growers predict yields based on berry weight and weather variable during the growing season.

Funding support: La Rioja Autonomous Government: FOMENTA program 2010/13

Use of Low-Dimensional Projection Techniques to Compare Ripening Evolution Based on Data from Wireless Sensor Networks

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The development of vineyard monitoring applications using wireless sensor networks has enormous potential benefit to help grapegrowers. This study evaluated three years (2009–2011) of data on soil moisture and temperature at multiple depths (30, 60, and 120 cm) and canopy temperature and humidity along the entire season. The goal was to correlate grape maturation with soil type over a range of environmental conditions. Two different soil types in the same 10 hectares vineyard were studied: one richer in sand and stone with fast drainage and low water-holding capacity and the other richer in silt and carbonates with a higher water retention ability. To analyze the complex data sets, multidimensional projections were used to graphically display correlations using linear (PCA) and nonlinear (Sammon) methods. Once the two-dimensional projections were done, the influence of soil and weather conditions on grape ripening could be studied over seasons. It is also possible to add seasons and compare the new projected points in the two-dimensional space with the other seasons stored in the database to reach conclusions on how grape ripening is evolving (pH, alcoholic grade, and tartaric acidity) during the present season.

Funding support: Autonomous Government of La Rioja FOMENTA-2010/13

Canker Diseases in the Coachella Valley: Incidence and Evaluation of Management Strategies

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Grapevine canker diseases such as Eutypa dieback, Esca, or Bot canker are caused by a complex of fungal pathogens. These diseases are a primary factor limiting vineyard longevity, productivity, and profitability. Following pruning wound infection, the pathogens progress slowly and vineyards that are over 10 years old start to express the disease symptoms (i.e., wood dieback). Recently, we noticed five-year-old table grape vineyards expressed the symptoms. We

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collected 60 wood samples from 11 vineyards ranging from 2 to 35 years old in the Coachella Valley, Riverside County. *Togninia minima, Phaeomoniella chlamydospora, Phaeoacremonium parasiticum, Phaeoacremonium fuscum, Lasodiplodia theobromae, Lasodiplodia crassispora,* and *Neoscytalidium dimidiatum* have been isolated. Fungi were identified to the species level with multigene sequence phylogenies and with morphological characters. The most effective way to control these diseases is to protect pruning wounds with fungicides. Our goal is to evaluate the long-term benefit of post-pruning thiophanate-methyl tractor applications as a preventative method to control these pathogens during the establishment phase of the vineyard.

Evaluation of the Grape Rootstock O39-16 as a Nematode Antagonist against *Xiphinema index*

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Xiphinema index is an ectoparasitic nematode that vectors grape fanleaf virus (GFLV), the causal agent of fanleaf degeneration. Grape roots can survive for many years after the removal of a vineyard and may serve as a food source allowing soil-borne pests to survive for long periods in fallow vineyards. The grape rootstock O39-16 is resistant to *X. index* feeding and prevents the expression of fanleaf degeneration. Currently, it is the only effective rootstock available to plant in areas infested by *X. index* and GFLV. Given its strong resistance to *X. index* feeding, O39-16 may be able to eliminate this nematode from infested soils. The purpose of this research is to evaluate the status of *X. index* in vineyards that have been planted on O39-16 for at least 10 years. The study site was located at Rutherford, California, and was planted in 1996. The GFLV status of the vines was determined using RT-PCR. Soil samples were collected from beneath the drip emitters of infected vines to maximize the chances of *X. index* recovery. Nematodes were extracted from the soil samples using Cobb's sieving method followed by extraction with Baermann funnels and visual identification. If O39-16 is able to eliminate *X. index* from infested soils, growers would have far greater flexibility in their replanting decisions. More importantly, it would break the link in the transmission of GFLV and eradicate fanleaf degeneration.

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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Rootstock Effect on Vegetative Growth, Yield, and Fruit Composition of Norton (*Vitis aestivalis*-derived) Grapevines

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Norton is an important commercial grape cultivar commonly grown in Missouri and the surrounding region known for its high wine quality and excellent disease resistance. However, Norton poses many challenges by producing fruit with high levels of pH, malic acid, and juice potassium which are known to reduce quality, aging potential, and stability of wine. Additionally, Norton tends to have an overly vegetative growth pattern. A potential solution to these traits is the use of rootstocks. The early effects of selected rootstocks on Norton fruit composition, yield, and vegetative growth were studied in St. James, Missouri, within a commercial vineyard planted in 2004. Rootstock treatments selected were 3309C, 101-14, Schwarzmann, 5BB, SO4, 1103P, 110R, 140Ru, 1616C, 44-53M, and own-rooted Norton as a control. Data collection was taken in years 2010 and 2011. Rootstock had little to no effects on vegetative growth and fruit characteristics. However, rootstock did influence yield, juice minerals, and petiole nutrient content. Rootstock 110R had the highest yield and generally greatest Ravaz index and own-rooted Norton the lowest overall. This was reflected in cluster weight, clusters per shoot, and to some extent berry weight. Juice minerals N, Mn, and B as well as petiole nutrients P and K were affected by rootstock and year. Generally, year impacted nearly all parameters measured, which may be attributed to seasonal differences and harvest decisions made based on pH by the cooperator. More extensive research is needed to determine the long-term effect of rootstocks on Norton grapevines.

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

Undervine Cover Crops Reduce Titratable Acidity without Affecting Vine Growth in Cool-Climate Riesling

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Given the rising concerns of herbicide resistance, runoff, and environmental contamination coupled with increased consumer desire for sustainably produced wine, investigating alternatives to herbicide use in vineyards is warranted. In the northeastern United States, cover crops are generally grown between rows while an herbicide strip is maintained to reduce competition for water and nutrients under the vines. Annual species of cover crops planted directly underneath Riesling (cl. 110/9)/3309C vines were evaluated as an

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alternative to an herbicide strip in the cool climate of the Finger Lakes in New York. Cover crops of buckwheat (*Fagopyrum esculentum*) at 348 lbs/acre and chicory (*Cichorium intybus*) at 5 lbs/acre were planted in a 1 m strip directly under rows at the start of the growing season and compared to a weed-free strip maintained with glyphosate. Irrigation was implemented to alternating halves of rows in a split-plot overlay in order to determine the effects of water availability on undervine covers. No differences were found among treatments throughout the season in stem water potential, cane diameter, shoot growth rate, shoot tip activity at veraison, or harvest yields in the first year of the experiment. The undervine cover crops had no consistent effect on the Brix or pH of juice, but titratable acidity was reduced by up to 1.0 g/L in grapes grown with an undervine cover crop compared to the control. These results indicate that the undervine cover crops improved fruit quality by lowering titratable acidity, but had no impact on overall vine growth or yield compared to the conventional herbicide strip. Continuing this experiment to see multiple-year effects and conducting sensory evaluation of wines resulting from the different treatments will help further evaluate the potential of undervine cover crops as a sustainable alternative to herbicide use.

Funding support: The Ronni Lacroute Dean's Discretionary Fund for Enology and Viticulture at Cornell University

Pruning Systems and Canopy Management Practice Interact on the Yield and Fruit Composition of Syrah

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A production trial in the San Joaquin Valley (SJV) of California was conducted where canopy microclimate of Syrah05/SO4 grapevines was altered through three pruning systems and two leaf removal treatments arranged factorially to rejuvenate vineyards with declining productivity. Vines were either pruned by hand to 44 nodes each, mechanically box-pruned to a 10 cm hedge, or cane pruned by hand to six 8-node canes arranged in opposing directions of the row with horizontal canopy separation. Outer surface layer of leaves were removed mechanically 20 days postbloom on the east side of the canopy in a 45 cm zone above the cordon in the fruit zone, or not. Yields from spur and mechanically box-pruned vines were considered too low for the study area, and leaf removal had no effect on yield components. Spur-pruned vines reached 24 Brix earlier than mechanically box-pruned and cane-pruned vines, in each year. Leaf removal had no effect on fruit composition of Syrah at harvest. Berry skin phenolics were not consistently affected by treatments applied. Cane pruning resulted in 3.8 leaf layers, with 32 shoots per 30 cm of row, 7.77 kg/kg Ravaz index, and consistently ripened 22 tons/ha to 24 Brix and should therefore be

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used in the SJV to improve yields in vineyards with declining productivity. The study identified a pruning system for vineyards in warm climates that can sustain yields and provides management information for growers on how to rejuvenate vines that have declined in productivity.

Funding support: American Vineyard Foundation, Bronco Wine Company Research Chair Trust

A Review of a Replicated Nine Variety Winegrape Trial in Round Mountain, Shasta County, California

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Nine own-rooted winegrape clones—Zinfandel, Merlot, Syrah, Sangiovese, Pinot noir, Pinot blanc, Cabernet Sauvignon, Chardonnay, and Sauvignon blanc—were planted in a replicated trial on an unclassified red clay loam at 2,855 ft. elevation on 1 May 1999. The vines were poorly managed until 2009 when new ownership took over management of the vineyard. Overall yields averaged 3.3 tons/acre for a 9/28 to 10/6 harvest. Brix sugar increased 0.51% per day in 2012. Zinfandel had significant immature fruit at harvest and Sangiovese had high yields but low sugar, suggesting either overcropping and/or late maturity. Preferred varieties at this location in 2012 were Chardonnay, Cabernet Sauvignon, and Merlot. Acceptable varieties at this location in 2012 were Syrah and Sauvignon blanc.

Funding support: Shasta and Lassen Counties Cooperative Extension

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Crop Water Use in Two Different Regions across California

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A crop water use analysis was performed at critical phenological stages during the 2011 growing season in two different regions across California. Crops were remotely identified using the crop data layer (CDL) from the National Agricultural Statistics Service (NASS) and a large amount of ground data. Accuracy assessment for the CDL was performed on the basis of visual identification of a randomly selected number of fields, within each growing region. The CDL crop layer was further digitized to provide crop identification at the field level. Pixels were grouped using the thematic grouping function from ERDAS Imagine software and field boundaries in order to create a crop layer, which was then used to estimate water use on a field basis. Estimates of real time water use (ETa), crop water stress (ks) and potential water use (kcp) were obtained by running the surface energy balance algorithm at the land level (SEBAL) at three critical stages during crop development. Results were grouped by crop, focusing on the main crops within the two different regions. Crops such as alfalfa, almonds, cotton, cherries, grapes, pistachios, olives, and walnuts were included on the analysis. Results showed significant differences among crops for all the variables analyzed and highlight the importance of these tools to estimate water use at a macro level. A water productivity analysis, using the ration between yield (tons/acre) and water used, was performed for grapes only and showed significant differences between regions and within vineyards in a single region. Future work will focus on extending this analysis to all grape-growing regions across California.

Funding support: E & J Gallo Winery

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Spatial and Temporal Water Use Variability in a Cabernet Sauvignon Vineyard in California

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A field trial was conducted during 2012 in a *Vitis vinifera L.* cv. Cabernet Sauvignon commercial vineyard located in the Central Valley of California. Vines were drip irrigated at standard commercial irrigation level starting in early summer when shoot growth rate had decreased significantly. Trellis/training system, clonal selection, rootstock, and vine management practices were identical for all vines in the vineyard block. The purpose of this trial was to study spatial and temporal variability in vine water use, while also looking at spatial variability in canopy temperature. Ground, continuous measurements of sap flow and above-canopy leaf temperature were performed in eight locations within the vineyard. Energy balance models were run using satellite imagery (Landsat TM7) in order to obtain estimates of basal crop coefficients (kcb) and basal evapotranspiration (ET_c) for the vineyard throughout the growing season. Leaf area index (LAI) was mapped from a large number of geo-referenced measurements taken on a grid pattern across the vineyard. Normalized difference vegetation index (NDVI) was calculated from aerial multispectral images and used to better understand the variability observed in water use. Yield was mapped using data from yield monitors mounted on mechanical harvesters. Fruit composition was measured from sampling locations defined by NDVI and LAI variability. Up to two-fold significant differences in vine water use within areas of the vineyard were found. These differences correlated well with the variability in NDVI, LAI, and yield. Correlations among the above variables are discussed from the standpoint of water use.

Funding support: E & J Gallo Winery

Enhancing the Growth of Grapes Grafted on Dwarfing Tetraploid Rootstocks with Plastic Shelters

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Colchicine-induced autotetraploid rootstocks, which have thicker and shorter fine roots and a more compact root system than the original diploid rootstocks, are used to induce moderate water stress on scion cultivars and improve the berry quality in the wet and warm climate of Japan. However, grapevines grafted on these tetraploid rootstocks grow more slowly than those grafted on the original rootstocks (diploids) in the nursery. To enhance growth in the nursery and shorten the period of establishment in the vineyard, polypropylene shelters and polyester mesh shelters were used to cover the grapevines.

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Shoot growth and root growth of Cabernet Sauvignon and Pinot noir grafted on diploid and tetraploid versions of Kober 5BB and Riparia Gloire de Montpellier with or without shelters were compared. The plastic shelter and mesh shelters increased shoot growth (shoot length, number of nodes, node length, and total leaf area) and the root growth across all rootstock types with almost constant shoot-root rates, suggesting that growth was balanced between shoot and root. The plastic shelters increased air and leaf temperatures and relative humidity, but decreased light intensity, CO_2 concentration in the shelter, and reduced the transpiration conductance and photosynthetic rate of leaves. Reduction of light intensity enlarged leaf area and reduced specific leaf weight and the ratio of stem diameter to shoot length of the grapevines. By protecting the vines from wind and maintaining humidity, the plastic shelter may increase water use efficiency. Increasing total leaf area without water stress in a plastic shelter may compensate for the reduced photosynthetic rate. As a result, using plastic shelters to enhance shoot and root growth of grapevines grafted on the dwarfing tetraploid rootstocks may induce sufficient growth to hasten transplanting of these vines in the vineyard.

Funding Support: JSPS

Developing Tools and Resources for Clonal Identification in *Vitis vinifera*

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We are currently developing an efficient method for the genetic identification of clonal variation in *Vitis vinifera*, and creating an online resource to provide a centralized resource for information regarding winegrape clones. Utilization of clones in the vineyard and winery is a valuable resource, but currently there is no straightforward and reliable method for the certain identification of individual grape clones. At present, growers must be confident that the identification of a particular clone has been properly documented since the original propagation or simply wait until the vine has matured to determine if it is true-to-type. In order to benefit from currently available knowledge regarding clones, it is essential to reliably identify varieties and their clones. Our first approach for developing a method for the genetic identification of clones involved a genetic marker technique that had not yet been tested in *V. vinifera*. Although we were able to visualize clear differences between the tested varieties, we found very limited differences between the clones and none allowed for the unique identification of the clones tested. Our current strategy is to focus on the *V. vinifera* transcriptome, with a sequence-based approach to analyze actual base pair polymorphisms in selected sequences of expressed DNA. A counterpart to the need for reliable identification of clones is an understanding of clonal differences in grape composition, vine morphology, and

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vine response to abiotic and biotic influences. This motivated the development of a centralized resource for the wide range of information that is available regarding winegrape clones. An internet database is being developed to compile details about clones from sources such as ENTAV-INRA in France, CSIRO in Australia, FPS in California, nurseries, growers, and winemakers.

Funding support: Washington State University Viticulture and Enology Program

Comparison of the Anatomical Variation among Drought Resistant and Susceptible Rootstocks

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The distribution of anatomical tissues is significantly different among rootstocks that vary in their level of drought resistance. Preliminary observations found that Ramsey and Riparia Gloire, two rootstocks placed at opposite ends of the drought resistance spectrum, have significantly different xylem tissue. These differences may have a direct correlation to the mechanisms employed by drought resistant individuals. Here we report on a survey of five rootstocks and three accessions currently being used in rootstock breeding: 5C, 110R, 101-14 Mgt, St. George, 420A Mgt, *Vitis girdiana,* and two *Vitis arizonica* accessions. Three woody root replicates were collected from each individual during the month of January 2012. These were preserved, sectioned, stained, and photographed for tissue quantification. Photoshop measuring tools are being used to determine the total area inside each xylem vessel (vessel lumen) and measure the rays, xylem fibers, and cortex. Data collected to date support the trend of smaller more abundant vessels in drought resistant individuals (Ramsey and *V. arizonica*). Complete results will be presented in relationship to the expected drought tolerance of the studied rootstocks.

Funding support: California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, American Vineyard Foundation, CDFA Improvement Advisory Board, California Table Grape Commission

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The Effects of Sunlight and LED Light on Methoxypyrazine Content of Ripening Berries of Cabernet Sauvignon

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Vegetal aromas and flavors resulting from methoxypyrazines (MPs) in grapes and wine can contribute to varietal character in a positive manner and in excess they can be unpleasantly herbaceous and detract from fruity aromas. Previous research demonstrated a relationship between light exposure and MP levels in fruit; however, it is difficult to distinguish irradiance effects from consequential temperature effects. This study attempted to separate the effects of light exposure and heat on berry MP with a novel treatment to increase light levels of shaded fruit through use of minimal heat-emission light-emitting diodes (LED). Field experiments in 2012 exposed developing Cabernet Sauvignon clusters to three treatments: full sun, full shade, and full shade with supplemental LED lights. Treatment conditions were created and maintained by shoot positioning. Fifteen vines were selected for each light treatment in both preveraison and postveraison periods, with one cluster per vine receiving a treatment. Accumulation and degradation of two prominent MPs—3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP)—were measured in fruit from each treatment. While in the field, photosynthetically-active radiation (PAR) measurements were taken weekly. Clusters were collected 50 days postanthesis and then at harvest when Brix was approximately equal to 24. Stir-bar sorptive extraction-gas chromatography-mass spectrometry (SBSE-GS-MS) was used to quantify IBMP and IPMP in grape samples. In preveraison and postveraison, the shade treatment PAR was significantly lower than both full sun and LED. However, in postveraison, the LED treatment PAR was 9% lower than the full sun treatment. For preveraison fruit, IPMP was lower than the limit of detection, and while IBMP was detected, it did not differ among treatments.

Funding support: Texas A&M AgriLife Extension Service

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A Leafhopper-Transmissible Geminivirus Is Present in Grapevines Showing Redleaf Symptoms

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A graft-transmissible disease with foliar symptoms resembling the grapevine leafroll disease, tentatively designated as grapevine redleaf disease (GRD), was observed in own-rooted Merlot and Cabernet franc grapevines (*Vitis vinifera*). Yield measurements at the time of commercial harvest showed 22% and 37% less fruit in GRD-affected Merlot and Cabernet franc grapevines, respectively, when compared with corresponding non-symptomatic grapevines. An analysis of fruit quality attributes indicated that berries from GRD-affected grapevines of both cultivars had significantly lower total soluble solids (12% in Merlot and 14% in Cabernet franc), higher titratable acidity (9% in Merlot and 16% in Cabernet franc), and lower total extractable anthocyanins (4% in Merlot and 9% in Cabernet franc) when compared to berries from non-symptomatic vines. In contrast, no significant difference was observed in pH of juice extracted from berries of symptomatic and non-symptomatic grapevines of both cultivars. Next-generation sequencing of high-quality total RNA obtained from symptomatic and non-symptomatic leaves was used to identify etiological agents present in grapevines showing GRD symptoms. A single-stranded DNA virus, tentatively named Grapevine-infecting geminivirus (GiGV), and *Grapevine fanleaf virus* were detected only in grapevines showing GRD symptoms. In contrast, *Grapevine rupestris stem pitting-associated virus, Hop stunt viroid, Grapevine yellow speckle viroid* 1, *Citrus exocortis viroid,* and *Citrus exocortis Yucatan viroid* (CEYVd) were present in both symptomatic and non-symptomatic grapevines. Molecular and phylogenetics analyses indicated that GiGV represents an evolutionarily distinct lineage in the family *Geminiviridae* with genome characteristics different from other leafhopper-transmitted geminiviruses. GiGV was transmitted by the Virginia creeper leafhopper (*Erythroneura ziczac* Walsh) from grapevine-to-grapevine under greenhouse conditions. To the best of our knowledge, this is the first report of the transmission of a grapevine-infecting ssDNA virus by a leafhopper species in the genus *Erythroneura*.

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Shifts in Fruitfulness and Crop Load of Pinot noir in Response to Nitrogen Depletion

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Oregon Pinot noir vineyards are generally characterized as having low crop loads given the large canopies relative to naturally low yields. Premium Pinot noir producers commonly reduce crop load further by fruit thinning. The long-term implications of these practices on vegetative growth, fruitfulness, fruit set, and berry quality are not fully understood for low yielding, cool-climate situations. During 2011 and 2012, a study was conducted in a commercial vineyard to assess the interaction of nitrogen (N) status and yield on vegetative and reproductive growth. Canopy size was manipulated through vineyard floor management practices that influenced vine tissue N through use of competitive perennial grass and/or tillage. Yield was manipulated as a split-plot with full and half crop levels. Petiole and leaf blade tissue samples measured at bloom and veraison each year were significantly lower in the grass treatment (lowest %N) resulting in reduced leaf area compared to tilled. The number of lateral shoots and lateral leaf area decreased 69% with the presence of grass at veraison in 2012. Stem water potential was not different among treatments across the growing season, indicating N as the limiting growth factor. Although there was an increase in canopy sunlight penetration by up to three-fold in the lowest N vines, the number of inflorescences per shoot was 11% lower than the higher N vines in 2012. The number of florets per inflorescence was reduced 10 to 35%, but fruit set increased 18 to 29%. Cane weights in the highest and lowest %N were 109 g and 55 g, respectively, resulting in a range of crop load from 0.98 to 3.40. Biomass (leaf area and pruning weight) was influenced more by N status than crop level. This work aims to further understand source-sink relationships to define optimal relationships between vegetative growth, yield, and fruit quality.

Funding support: Oregon Wine Board and Northwest Center for Small Fruits Research

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Kaolin-Based Foliar Reflectant Alleviates Heat Stress in Deficit Irrigated Malbec

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We evaluated the interaction effects of a kaolin-based particle film and water deficit on leaf and berry surface temperature, light reflectance, gas exchange characteristics, berry composition, and yield of Malbec vines under field conditions over three growing seasons to test the hypothesis that the foliar reflectant would increase anthocyanins and water use efficiency and that response would be less effective under increasing water stress. Own-rooted Malbec grapevines were irrigated with a standard or reduced amount of water and grown under arid field conditions with high solar radiation. Particle film increased the concentration of total monomeric anthocyanins in berries each year and the ratio of anthocyanins to soluble solids in two out of three years. The particle film did not alleviate differences in anthocyanin concentration between east- and west-exposed clusters. Under reduced irrigation, particle film decreased the cumulative minutes that surface temperatures exceeded 30°C between mid-July through harvest in east- but not west-exposed berries and in east-exposed leaves. Leaves with particle film had lower midmorning rates of assimilation, lower chlorophyll-a to chlorophyll-b ratio, and a higher ratio of chlorophylls to carotenoids than leaves without particle film. Decreased assimilation was unrelated to the magnitude of leaf reflectance of visible light. Particle film did not affect yield components or fruit maturity, indicating that net primary productivity was sufficient to ripen fruit to maturity. These results demonstrate that foliar particle film can facilitate the accumulation of anthocyanins in deficit-irrigated Malbec under warm, arid conditions with high solar radiation.

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Economic Impact of Eutypa Dieback on *Vitis vinifera* cv. Cabernet Sauvignon Grown in Napa County, California

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Eutypa dieback is a serious grapevine trunk disease and is a major concern wherever Cabernet Sauvignon is cultivated worldwide. The economic impact can be severe and in this study its impact on Cabernet Sauvignon grown in Napa Valley was approximated. In this study, a net present value approach (NPV) was employed over a 25-year vineyard lifespan. The economic impact of yield reduction and shortened vineyard life as well as the benefits of two management options (active annual and retraining) were determined. If the disease is not controlled, the economic impact is estimated to be $14,970 per acre. Replanting a vineyard after 20 years due to large incidence (>40% infection) results in a loss

of $19,603 per acre. Given that the presence of Eutypa and other trunk diseases is ubiquitous in Napa, vineyard managers must actively adopt cultural practices and apply fungicides to protect vineyards. Active management results in a loss of $4,215 per acre or 15% of NPV compared to no treatment if this cultivar was not susceptible to the disease. Another management option, retraining of suckers on infected vines after 18 years of production, will result in a loss of $5,200 per acre. Grapes prices would have to be increased from 4 to 19% in order to offset losses due to Eutypa. The results show that the most significant impact that Eutypa may have on vineyards is due to shortening lifespan.

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Leaf Removal in Cool Seasons Enhances Pinot noir Anthocyanins

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Pinot noir grapes were harvested from a control (no leaf removal) and three complete leaf removed cluster zone treatments, which were initiated at three separate preveraison growth stages (bloom, grain-pea size, and bunch closure). Control and leaf-removed treatments were monitored and maintained until harvest for four growing seasons (2008–2011). The two most recent vintages (2010 and 2011) were evaluated for fruit quality and were considered cooler than normal Oregon growing seasons. Experiments were conducted at two commercially operating Oregon vineyards (Willamette Valley AVA; site A = Pinot noir 115/ 420A and site B = Pinot noir 777/ 3309C). Fruit maturity indices, anthocyanin composition, and tannins were examined at harvest. Leaf removal initiated at bloom and maintained until harvest produced maximum anthocyanin accumulation in Pinot noir grapes (site A = 85.24 mg/100 g and site B = 125.06 mg/100 g) compared to no leaf removal (control; site A = 57.91 mg/100 g and site B = 97.56 mg/100 g). Leaf removal did not alter most fruit maturity indices (berry weight, pH, and % soluble solids), and whole berry tannin levels were similar to no leaf removal (control). Even the last leaf removal initiation period (bunch closure) increased grape anthocyanin (site A = 73.22 mg/100 g and site B = 118.93 mg/100 g) compared to control, but total anthocyanins were lower than grapes from bloom leaf removal. If Oregon wine-grape growers desire the highest achievable levels of anthocyanins in their Pinot noir grapes during cool growing seasons, leaf removal initiated at bloom and maintained free of leaves until harvest is recommended. If leaf removal around bloom is not possible due to labor availability, similar results can be achieved by removing leaves between bloom and bunch closure to increase Pinot noir anthocyanin accumulation.

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Viticulture – CONTINUED

Compost Application Improves Cabernet Sauvignon Yield, Pruning, and Berry Weight without Changing Vine Balance

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Application of composted steer manure (CSM) was examined in the Red Hills AVA of Lake County, California, as a method to enhance productivity on historically underperforming portions of a vineyard. The intent of the research was to identify a dose response relationship between the application rate of compost, and vine nutrient status, biomass, and yield and to identify vineyard nutrient management strategies for underperforming vineyards in volcanic soils, such as the soils of the Red Hills AVA. A field study using a randomized complete block design was implemented to investigate vine response to incremental increases in CSM application rate (5, 10, and 15 tons/acre applied in December 2011) on the nutrient status, yield, pruning weight, and juice chemistry of Cabernet Sauvignon vines grown on 3309-C rootstock. Vine nutrient status was measured in petioles at bloom, veraison, and postharvest. Brix, titratable acidity, pH, berry weight, and per vine yield were measured at harvest. Pruning weights were measured January 2013. Nitrogen and phosphorus nutrient status at bloom displayed a significant linear response to CSM application rate. Nitrogen status was also significantly increased at veraison. Yields, pruning weight, and berry weights were significantly increased by application rate. Average per vine yields improved 50% between the control and high application rate. Yields, pruning weights, and berry weights all showed a significant linear response to compost application rates. The Ravaz index (yield/pruning weight) was not significantly affected by compost application, implying that, although yields increased, the CSM application did not result in significant changes to vine balance. Brix, titratable acidity, and pH were not significantly affected by compost application rate. This study demonstrates that compost application can significantly increase vine nutrient status, yield, pruning weight, and berry weight without changing vine balance or juice chemistry in vineyards in underperforming volcanic soils in northern California.

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Viticulture – CONTINUED

Postharvest High Heat Treatment on Grapes and the Effects on Wine Quality Related Compounds

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This research evaluated simulated accumulated summer temperature conditions in grape shipping containers during transportation from vineyard to winery in Texas. Temperature treatments were applied to five batches of destemmed grapes (three batches of red varieties and two batches of white varieties). After 0 to 15 hr treatments, the berries were processed and fermented to wines in microscale. Phenolic constituents important to wine-quality sensory perception, including total phenolics, anthocyanins, tannins, and polymeric pigments as well as color intensity, hue, and standard wine parameters were quantified to evaluate the effects of heat at postharvest stage on the subsequent wines. The results indicate that long-distance transportation of berries in Texas may result in high phenolic extraction and elevated browning in white wines, while red wines tended to change red color intensity, tannin concentrations, and red pigment profiles, which might not affect red wines sensorily. Heat treatment did not affect pH, titratable acidity, ethanol concentrations, browning, total phenolic concentrations, or anthocyanin concentrations compared to the wines made from the low heat treated berries. Composition of phenolic constituents in red wines is relatively stable after the heat treatment before vinification.

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