63 SIXTYTHIRD NATIONAL CONFERENCE

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## TECHNICAL ABSTRACTS

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AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

## Oral Presentation Abstracts

### Wednesday, June 20



### Thursday, June 21



## Poster Presentation Abstracts

### Wednesday, June 20 and Thursday, June 21



**NOTE:** pp. 1-48 constituted the program section of the original printed *Program and Technical Abstracts* booklet.

*Abstracts are listed in the approximate presentation order as scheduled in the conference program. Abstracts are those submitted and accepted through the 2012 Call for Abstracts.*

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# 2012 National Conference Technical Abstracts

**CONTINUED**

## Alphabetical Listing of All Authors with Submitted Abstracts

## Enology — Microbiology Session

### Impact of Oxygen Alterations in Chardonnay Juice on Fermentation Behavior with Different Yeast Strains

**Lauren A. Schwartzburg** and Linda Bisson*
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In order to assess the impact of prefermentation alterations of oxidative conditions in Chardonnay juice, 25 small-scale fermentations were carried out. Treatments varied in oxidative aerations or deficits and five different yeast strains were used. The oxidative conditions of the juice were altered at various points in the fermentation to determine optimal processing conditions. The treatments ranged from aeration or sparging with nitrogen prefermentation to aerations at a specific time point in the fermentation or postfermentation or receiving no intentional oxygen introduction. These treatments were intended to mimic realistic production practices in winemaking. Dissolved oxygen readings of the various conditions indicated negligible impact of oxygen introduction or mixing to the juice. Dissolved oxygen readings ranged from 0.05 to 0.12 mg/L and did not stabilize efficiently. Several factors could have influenced this observation, including enzymatic activity of the juice or sensitivity of the instrumentation. The yeast strains used included three *MET10*-932 Phyterra strains (P1Y0, P2Y3, and P7Y9) and two strains that contained the wild type *MET10* gene (CY3079 and EC1118). Preliminary results indicated a significant difference in the fermentation rate of P7Y9 from all other strains used, with P7Y9 taking an average 10 days longer than all other strains to complete primary fermentation. An additional benefit of this research was the examination of the *MET10*-932 yeast strains that have proven in a laboratory setting to be low in producing $H_2S$. As Chardonnay juice has historically produced $H_2S$ in primary fermentation, application of the yeast strains proved to be effective in determining fermentation capabilities of these newly identified strains. Significant differences among treatments within the same strain were not observed in any of the strains in the context of fermentation rates. This practical application is useful in aiding industry decisions in prefermentation processing treatments.

*Funding support:* University of California, Phyterra, and Jordan Vineyard and Winery

## Enology — Microbiology Session – CONTINUED

### *Saccharomyces cerevisiae* Population Dynamics during Inoculated and Spontaneous Fermentations at Three British Columbia Wineries

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The population dynamics of *Saccharomyces cerevisiae* wine yeast strains during inoculated and spontaneous fermentations were examined in three wineries located in the Okanagan Valley of British Columbia, Canada. Nine guided and three spontaneous fermentation tanks containing *Vitis vinifera* L. Pinot noir berries were sampled at four distinct stages of fermentation (cold-soak, early, mid, and end). A total of 720 yeasts were isolated and identified from the 2010 harvest year. *Saccharomyces cerevisiae* strains were distinguished using PCR amplification of six hypervariable trinucelotide microsatellite loci and identified upon comparing their genetic fingerprints to a published comparative microsatellite active dry yeast (ADY) database and an ADY database specific to each winery. DNA sequence analysis of the internal transcribed spacer (ITS) and the D1/D2 domain regions of the large subunit of ribosomal DNA were used to identify non-*S. cerivisiae* spp. Twelve commercial *S. cerevisiae* strains and 20 unique *S. cerevisiae* fingerprints, or "unknown" strains, were detected from all three wineries. The detection of unknown *S. cerevisiae* isolates may be due to recombination events in commercial strains generating hybrid *S. cerevisiae* isolates or they may be of natural origin. Non-*Saccharomyces* species were the dominant yeasts in the cold-soak stage and *S. cerevisiae* strains were mainly isolated during early, mid, and end stages of fermentation. At two of the three wineries, the commercial *S. cerevisiae* starter cultures were not necessarily the dominant or finishing strain, and a noninoculant commercial strain, Lalvin ICV-D254, was consistently detected in both guided and spontaneous fermentations. The dominance of Lalvin ICV-D254 appeared to correlate with whether the winery had used this strain in fermentations of other grape varietals. At only one of the three wineries did spontaneous fermentation have a substantial strain diversity increase compared with the inoculated fermentations.

*Funding support:* Natural Sciences and Engineering Research Council and Quails' Gate Estate Winery

**Enology — Microbiology Session – CONTINUED**

## Effect of Yeast Strain on the Metabolism of $SO_2$-Binding Compounds and $SO_2$

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Although crucial for stabilization, there is a trend to minimize wine $SO_2$ concentrations to reduce potentially negative consumer health effects and perception. This can be achieved by limiting the presence of $SO_2$ binding compounds in wines before stabilization. The metabolism of major $SO_2$ binders (acetaldehyde, pyruvate, α-ketoglutarate, and acetoin) by yeast was studied. Two new commercial strains, a *Torulaspora delbrueckii* strain and a non-$H_2S$ forming *Saccharomyces cerevisiae* strain (P2Y3), were compared to the common *S. cerevisiae* strains EC1118 and CY3079. A New York Chardonnay was used in an anaerobic chamber to prevent oxidation. $SO_2$ binders were analyzed by HPLC following DNPH derivatization. Other metabolites were detected by HPLC and FT-NIR. Yeast viability was quantified by flow cytometry. All *S. cerevisiae* fermentations reached dryness within two weeks. The *T. delbrueckii* fermentation got stuck after 18 days and was then reinoculated with EC1118. Final α-ketoglutarate concentrations trended higher in *S. cerevisiae* fermentations (20 to 32 mg/L). Acetoin was not detected in any *S. cerevisiae* treatments but reached 2.4 mg/L in *T. delbrueckii* incubations. There was no clear trend for pyruvate, which ranged from 23 to 36 mg/L. Acetaldehyde, the most important $SO_2$ binder, was found at very low concentrations after growth of *T. delbrueckii*
(<5 mg/L but 20 mg/L after the second fermentation by EC1118). Strains EC1118 and CY3079 led to intermediate concentrations (~30 mg/L), while fermentation with strain P2Y3 resulted in acetaldehyde residues exceeding 50 mg/L. The total final $SO_2$ concentrations reflected these differences and were <5 mg/L for *T. delbrueckii*, 25 to 40 mg/L for EC1118 and CY3079, and >100 mg/L for P2Y3. The data suggest that yeast $SO_2$ production and viability in late fermentation phases correlated with acetaldehyde production and reuse, respectively.

*Funding support:* New York Wine and Grape Foundation

## Enology — Microbiology Session – CONTINUED

### Isolation and Identification of Non-*Saccharomyces* Yeast with ß-Glucosidase Activity and Their Impact on Pinot noir Wine Aroma

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Non-*Saccharomyces* yeast populations and diversity during prefermentation cold maceration and alcoholic fermentation of Pinot noir grapes and their impact on Pinot noir wine aroma were investigated. Yeast populations were followed during prefermentation cold maceration and alcoholic fermentation of Pinot noir grapes from a commercial vineyard in Dayton, OR. Fermentations were conducted at the Oregon State University research winery in 100-L tanks and grapes from the same vineyard lot were fermented at a commercial winery. Grapes were not inoculated for alcoholic fermentation. Samples were taken daily during prefermentation maceration (9°C) and alcoholic fermentation (27°C) and plated on WL and lysine media to determine *Saccharomyces* and non-*Saccharomyces* populations and diversity. Total non-*Saccharomyces* populations increased from 1 x $10^3$ cfu/mL to 1 x $10^5$ cfu/mL during prefermentation cold maceration and reached a maximum 1 x $10^7$ cfu/mL during alcoholic fermentation. Thirteen distinct non-*Saccharomyces* species were tentatively identified based on appearance on WL media. DNA sequence analysis of the D1/D2 domain is underway to confirm yeast species. Yeast were initially screened for β-glucosidase activity using 4-methylumbelliferyl-β-D-glucopyranoside plates. β-Glucosidase activity was further characterized and quantified using a liquid media representing grape must conditions (pH 3.5, 20 Brix) at 25°C or 8°C. While increasing sugar concentration suppressed β-glucosidase activity in *Hanseniaspora uvarum* (-99%), activity still remained relatively high for *Metschnikowia pulcherrima, Hansenula anomala*, and isolate #10. At 8°C, β-glucosidase activity in *M. pulcherrima* was less than at 25°C, but activity increased for *H. uvarum, K. thermotolerans, H. anomala,* and isolate #10. Non-*Saccharomyces* isolates demonstrating β-glucosidase activity were used to ferment Pinot noir grapes treated with high hydrostatic pressure to inactivate naturally occurring yeast and bacteria. All non-*Saccharomyces* isolates grew during cold maceration (9°C) by 3 to 4 logs. Following cold maceration, all ferments were warmed to 27°C and inoculated with *S. cerevisiae* RC212. Alcoholic fermentations were all complete within eight days and wines are currently being analyzed for volatile aroma compounds by SPME-GC-MS.

*Funding support:* Oregon Wine Board

**Enology — Microbiology Session – CONTINUED**

## Genetics of Ester Synthesis in *Hanseniaspora uvarum* during Fermentation of Wine

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Non-Saccharomycetes yeasts have a strong influence on the chemical composition and sensory quality of wines. *Hanseniaspora uvarum* is found in the early stages of spontaneous wine fermentations and also sometimes in early stages of inoculated fermentations. Up to 90% of the yeast population at the beginning of fermentation can belong to this species. In some cases, spontaneous fermentations with strains of this organism at the beginning show interesting bouquets caused by esters. However, fermentations with high populations of *H. uvarum* present during the beginning can be characterized by high acidic acid and the resulting ethyl acetate ester, both typical off-flavor compounds. In *S. cerevisiae*, esters are produced by two alcohol acyltransferases, ATF1 and ATF2, and an acyl-coenzyme A: ethanol O-acyltransferase EEB1. Which metabolic pathway(s) are responsible for esters produced by *H. uvarum* and how the relevant genes are regulated was studied. Genomic data for *H. uvarum* are not available so far. In a cooperative project, a type strain was sequenced. Using these sequences, possible candidate ATF and EEB genes in *H. uvarum* were identified and amplified by PCR. The PCR products were characterized by sequencing and cloned in *E. coli*. Corresponding EuroScarf knockout mutants were reconstituted, used to ferment grape must, and the ester formation was analyzed by GC-MS. In addition, the conditions under which high concentrations of several ester compounds were produced were correlated with high expression levels of ATF and EEB. The next steps will include the development of a transformation protocol, the generation of knockout and overexpression mutants, and analysis of the promoter sequences.

*Funding support:* Geisenheim Research Center

## Enology — Microbiology Session – CONTINUED

### Increased Complexity and Chardonnay Quality in Spontaneous Fermentation and Cofermentation with *Hanseniaspora vineae*

**Francisco Carrau**,* Karina Medina, Laura Fariña, Octavio Gioia, Maria Eugenia Gomez, Marianne Barquet, Carina Gaggero, Eduardo Boido, and Eduardo Dellacassa
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Searching for increased yeast biodiversity during winemaking is a goal for some winemakers who seek to differentiate and give particular characteristics to quality wines. This work analyzed the effect of applying a native apiculate yeast strain selected from grapes: *Hanseniaspora vineae* 02/5A. Sequential inoculation at industrial density with *Saccharomyces cerevisiae* resulted in dry Chardonnay wines with increased aroma and flavor complexity compared to the conventional fermentation with the commercial strain. Fermentations were carried out in 225-L oak barrels in triplicate with a Chardonnay grape must. Three different strategies were compared: conventional inoculation with commercial *S. cerevisiae* strain ALG 804, sequential inoculation with *H. vineae* and ALG 804, and a spontaneous fermentation. Yeast strain identification was performed at the end of the process and spontaneous fermentations were confirmed as finished by three new native *S. cerevisiae* strains. Basic winemaking parameters such as glycerol, biogenic amines, organic acids, and aroma compounds were determined by GC–MS. Sensory analysis was conducted by a trained panel and a group of winemakers. Wine fermented using sequential inoculation with *H. vineae* and *S. cerevisiae* was preferred by the expert winemaker panel due to its increased palate length and full body. This last descriptor may be due to a significant increase in glycerol and some aroma compounds in this treatment. The aroma sensory analysis by the trained panel cited increased tropical fruit character described as pear, pineapple, guava, and banana and some spicy notes of green tea, herbs, and dry fruit. The correlations with the GC chemical analysis, in which 16 compounds showed significant differences between treatments, are discussed in relation to their effect on aroma composition of the three treatments.

*Funding support:* Universidad de la Republica and Consejo Sectorial Grupo I+D 656

## Viticulture — Grape Anatomy Session

**Martin C. Goffinet*** (Keynote Speaker)
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### Lecture 1: Grapevine Vegetative Structure and Whole-vine Vascular Integration

To set the stage, this presentation will cover the annual cycle of shoot and root development at both the gross and microscopic levels and involves discussion of shoot-to-root vascular connectivity. The vine's major organs (leaves, tendrils, inflorescences, buds, roots, etc.) arise in specific locations (meristems) having high rates of cell division and cell differentiation in patterned sequences. Individual organs, tissues, and cell types mature to serve specific functions, which will be reviewed with respect to whole-vine development and integration. Several concepts applicable for discussion include the following: (1) The vine is generated from a system of shoot and root meristems in patterned formation (2) Specialized structures are inseparable from specialized function (3) Seasonal patterns of development are affected by prior year's development and this year affects next year's potential (4) Viticultural management both constrains and takes advantage of the vine's natural growth tendencies.

### Lecture 2: Grapevine Buds – Origin, Development, and Fruitfulness under Management

In many ways much of what is termed viticulture involves what may be termed "budiculture." This is because cropping in grapevines involves the initiation and development of buds with our anticipation of high fruiting potential for next season, while we must simultaneously manage shoots and fruiting clusters growing in the current season. This presentation and discussion will review the origin of buds from shoot apical and axillary meristems, the development of these buds and internal details through summer shoot growth, and the bud's preparation for winter dormancy. Included will be the origin and development of the inflorescence primordia and their subsequent development as flower/fruit clusters the second season. Examples taken from the presenter's experience will include influences of imposed stresses on bud and cluster formation and floral development, such as severe canopy treatments, carbohydrate limitations, vine size and crop load effects, as well as the importance of heat-unit accumulations bud-break to bloom. Applicable concepts for discussion include the following: (1) The bud is a condensed

axillary branch (2) Bud initiation, inflorescence initiation, and flowering/fruiting have multi-year factors to consider (3) Degree-day accumulations and carbohydrate and nitrogen reserves drive metabolism for bud-break, spring shoot emergence, and flower development.

### Lecture 3: Grapevine Tissue and Organ Injury, Vine Accommodation to Injury and Vine Repair Strategies

Grapevines sustain many nature-imposed and grower-imposed injuries. Vines have several levels of protection against tissue and organ injury, and in many cases will attempt to recover from or to circumvent such injury. Some injuries cannot be mended and affected organs will senesce and die. This presentation will look at examples of cell and tissue injury and some strategies the vine may take to repair, isolate, or by-pass injury. Response to cellular injury may include hypersensitivity, cell wall changes, exudation and accumulation of resistant materials, vascular plugging, etc. Organ injuries may invoke abscission, compartmentalization, renewal of cell division and callus production, wound periderm formation, and differentiation of bridging vascular tissues. Examples offered may include bud injury, vine cold injury, stem/trunk diseases, leaf and root injuries, viral/mycoplasm diseases, and adventitious rooting and graft-union formation viewed as repair responses. Discussion may include other examples offered by attendees. A key concept in vine response to injury is that of "totipotency," that is, the ability of cells to de-differentiate, losing former function, and to renew cell divisions for tissue repair.

### Importance of Anatomy to Hydraulic Physiology across Grapevine Rootstocks Subjected to Drought Stress

**Andrew J. McElorne**,* Greg A. Gambetta, Cecilia Osorio, and M. Andrew Walker
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Water scarcity will always threaten agriculture in the western United States and growers require ever-evolving tools to confront this challenge. New grapevine rootstocks with improved drought resistance would provide a tool to improve vineyard water use efficiency by maintaining yield/fruit quality while using less irrigation. Plants possess a variety of mechanisms to resist drought, but the mechanisms underlying drought resistance of grapevine rootstock material and whether they can be used effectively for selection criteria in a breeding program are currently not known. Using the Walker lab collection of *Vitis* accessions from arid southwestern United States, we

are evaluating the mechanisms that enable grapevines to tolerate the harsh growing conditions of their native habitats. We are combining the power of a variety of tools to determine structural and functional links involved in drought resistance and root water uptake physiology of grapevines. We are using (1) an advanced visualization technique (high-resolution X-ray computed tomography) with a micrometer-range resolution to capture continuous serial sections through woody grapevine roots and detect subtle differences in xylem anatomical contribution to hydraulic physiology and (2) laser-capture microdissection to pinpoint gene expression patterns linked to anatomical changes important for water uptake along the length of grapevine roots.

*Funding support:* National Science Foundation, American Vineyard Foundation, USDA-Sustainable Viticulture CRIS, and NIFA-Specialty Crops Research Initiative

## Multiple Compound Buds in *Vitis vinifera* L.

**Luis A. Sánchez*** and Nick Dokoozlian
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Grapevine buds are compressed shoots at the axils of leaves that begin to form as they separate from the apical meristem of the shoot (N shoot). Depending on order of appearance at a node and subsequent development, grapevine buds fall into three categories: (1) the prompt bud (N+1), which forms first and from which the summer lateral develops, (2) the primary bud (N+2), which forms at the basal node of the summer lateral, and (3) the secondary buds (N+3), which are borne opposite each other at the axils of the two basal prophylls of the primary bud. The N+1 shoot may grow or it may abscise above the prophyll, leaving a scar. The N+2 and two N+3 buds develop enclosed by the basal prophyll of the summer lateral and the two basal scales of the primary bud, respectively, and constitute the compound (latent) bud from which the following season's shoots grow. Although it is well known that only one compound bud develops at each node, dissections of dormant buds and in-season observations in 30 California winegrape vineyards over seven years have shown that the summer lateral (N+1) can produce two basal nodes separated by a highly compressed internode, each

**Viticulture — Grape Anatomy Session – CONTINUED**

bearing a compound bud complete with primary (N+2) and secondary buds (N+3). These multiple buds occur in the proximal nodes of the N shoot. This arrangement can be distinguished from normal buds by the phyllotaxy of the enclosed buds and by the absence of the N+1 scar at the usual location. The abundance of multiple buds is cultivar-dependent. Awareness of this arrangement is important for the assessment of fruitfulness through bud dissections.

*Funding support:* E&J Gallo Winery

## Xylem Anatomical Indices and Grapevine Vigor: A Mechanistic View

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To gain a mechanistic understanding of grapevine vigor, Merlot vines were pruned to a range of bud numbers, resulting in vines with different shoot morphology that primarily varied in shoot length, which was used as an index of vigor. Various xylem anatomical indices coupled with physiological measurements were recorded at specific phenological events for all vigor levels. Shoot length varied inversely with bud number. Shoot fresh weight, internode number, total leaf area, cluster weight, stomatal conductance, soluble solids, and nutrient levels increased with shoot length, whereas soluble solids decreased with cluster number. Regardless of vigor, the successive internode lengths and corresponding leaf area increased linearly at the proximal end, whereas the opposite occurred at the distal end (shoot tip). Shoot structure was modular: basal internodes without tendrils tended to be shorter, but developed more lateral branches. A significant relationship was found between internode length and leaf size above or beneath that internode. Vessel lumen diameter, vessel number, hydraulic conductivity, the number of radial sectors bordered by xylem parenchyma, and vessel number per radial sectors were smaller at shoot distal ends than at proximal ends, but vessel density followed the opposite trend. Vessel number per cross-sectional area, sapwood area, vessel lumen diameter, total vessel area per cross-section, and hydraulic conductance increased with shoot length, whereas vessel density decreased with shoot length. This study showed that when shoot length served as a vigor indicator, vigor was strongly related to hydraulic conductivity, which in turn was governed by various xylem anatomical indices.

*Funding support:* Northwest Center for Small Fruits

## Enology — Molecular Biology Session

### Establishment of Prions in Wine Strains of *Saccharomyces cerevisiae* as a Cause of Sluggish and Arrested Fermentations

**Gordon A. Walker** and Linda F. Bisson*
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An extreme preference for glucose and fructose as carbon source is a hallmark of *Saccharomyces cerevisiae* metabolism. This preference can be overcome in cells that harbor a novel, dominant, transmissible prion known as the [*GAR*+] (for "resistant to glucose-associated repression") phenotype. Prions are proteins capable of two conformational states, one of which is self-templating. Yeast prions are heritable, reversible, epigenetic "switches" that allow cells to quickly alter their phenotype in response to stressful environmental conditions. A cell harboring the [*GAR*+] prion is capable of utilizing alternative carbon sources such as glycerol or maltose despite the presence of glucose. We investigated the ability of several commercial and native wine isolates to enter the [*GAR*+] state. Wine strains varied from a frequency of 1 in 25 cells to virtually never inducing the [*GAR*+] prion. We then evaluated the impact of the [*GAR*+] prion on wine strains, fermentation progression, and ethanol tolerance. [*GAR*+] was induced in the UCD932 strain and then evaluated in Chardonnay fermentations as compared to the [*gar*-] parental strain. While the 932 [*gar*-] fermentation proceeded normally, the 932 [*GAR*+] fermentation displayed sluggish initiation and remained sluggish throughout fermentation, eventually arresting at 3 Brix. Microscopic observation of the [*GAR*+] fermentation lees revealed a plethora of lactic acid bacteria not found in the [*gar*-] control fermentation. We hypothesize that the establishment of the [*GAR*+] prion causes a conformation change in the major plasma membrane ATPase, Pma1. This [*GAR*+]-mediated conformational change reduces a cell's ability to effectively regulate pH homeostasis and consequently reduces the fermentative fitness of a cell by slowing amino acid uptake and sugar metabolism. We have also observed that certain common spoilage organisms can induce [*GAR*+] in wine yeasts at high levels, providing a possible mechanism for how some sluggish/arrested fermentations occur.

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## Enology — Molecular Biology Session – CONTINUED

### Metabolomic Analysis of $SO_2$ Tolerance and pH Effects on *Brettanomyces bruxellensis*

**C.M. Lucy Joseph**,* Iliana Vigentini, and Linda F. Bisson
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Metabolomic analysis was done on *Brettanomyces* strains under different growth conditions. Over 100 strains of *Brettanomyces bruxellensis* from the UC Davis Viticulture and Enology Culture Collection were screened for tolerance to sulfur dioxide. Two resistant strains were selected for further analysis, including metabolomic profiling. Strains that had previously been used to compare medium-grown and wine-grown metabolism were further analyzed to compare the metabolic profiles of the strains at different pH levels in wine. The metabolomics analysis was done at the Metabolomics Core Facility at UC Davis by GC-MS-TOF. Approximately 120 metabolites were identified from the *Brettanomyces* cells. Significant differences in metabolic profiles of sulfite resistant and sensitive cells were seen. The primary metabolic pathways affected by sulfite were amino acid pathways. Changes in carbohydrate and energy metabolism were also seen. An observed increase in arginine and proline metabolism may have wider implications as a survival mechanism for *Brettanomyces* in wine. Evidence indicates that proline can give some protection to a sulfite sensitive and a low tolerance strain of *Brettanomyces*. Added proline did not affect the sulfite tolerant strains. Plants and bacteria use proline to stabilize proteins under certain stress conditions. Some mechanisms that have been shown to protect *Saccharomyces cerevisiae* from exogenous sulfite do not appear to be active in *Brettanomyces bruxellensis*. Recent genetic sequence data indicates that *Saccharomyces* may not be a good model organism for *Brettanomyces* metabolism.

*Funding support:* American Vineyard Foundation and California Competitive Grant Program for Research in Viticulture and Enology

**Enology — Molecular Biology Session – CONTINUED**

## Genomic and Phenotypic Profiling of *Saccharomyces cerevisiae* Wine Yeast Shows Evidence of Admixed Populations

**Chandra Richter**,* Barbara Dunn, Gavin Sherlock, and Tom Pugh
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Louis Pasteur in the mid-1800s described the scientific basis for fermentation as being attributed to yeast sipping grape juice sugar and excreting alcohol. As the importance of yeast as an ingredient in winemaking became better appreciated, companies began to collect and market specific yeast strains to enhance positive and minimize negative attributes in wine. It is generally believed that each strain can contribute uniquely to fermentation performance and wine style because of its genetic background; however, the impact of genomic diversity among yeasts on aroma has not been comprehensively examined. Using custom-designed microarrays and replicated fermentation studies, we provide evidence of genome-wide diversity and profiles of metabolic traits in 70 commercial wine yeasts. This data set has identified interspecific hybridization events, introgression events, and copy number variation in all but a few strains. In addition, considerable phenotypic variation was seen, suggesting that the genome-wide variation has contributed to metabolic diversity. Interestingly, pervasive copy number variation was distributed throughout the strains and did not produce clear phylogeny. However, these data help identify genomic regions involved in adaptation to industrial winemaking. Correlations to specific metabolic traits have provided new insights into the metabolic individuality of wine yeasts. These data suggest that genetic and phenotypic evolution has occurred in these strains through environmental selection and/or human selection.

*Funding support:* E&J Gallo Winery

## Enology — Molecular Biology Session – CONTINUED

### Microbial Terroir of Wine: Deep Insight into Site-Specific Winery and Vineyard Microbial Communities

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While *Saccharomyces cerevisiae* is indisputably the primary microbial driver in wine production, wine fermentations involve surprisingly diverse communities of yeast and bacteria introduced from the vineyard and winery surfaces. The ecology and impact of minor yeasts and bacteria on grapes and in wine have recently received a great deal of attention, yet comprehensive ecological studies are hindered by the sensitivity and/or low throughput of traditional detection schemes implemented in such studies. We have adapted culture-independent tools based on terminal restriction fragment length polymorphism (TRFLP) and barcoded next-generation sequencing (NGS) for high-throughput profiling of yeast and bacterial communities of wines, musts, and grape surfaces with improved sensitivity for deep resolution of resident microbiota. These tools achieve rapid characterization of microbial communities, enabling large, multivariate studies of global ecology. We used a mixture of these methods to study the complex yeast communities and "rare microbiome" of botrytized wine fermentations. We also used NGS of 270 press-pan samples from eight wineries across California to explore how the patterns of microbial residents in vineyards and wineries constitute a "microbial terroir" unique to that site. These novel tools create an unprecedented opportunity to comprehensively explore the diverse microbial communities inherent in the complete grape-to-wine continuum.

*Funding support:* Oregon Wine Board California Competitive Grants Program for Research in Viticulture and Enology

## Enology — Sensory Session

### Putting the Texture Back into White Wines: Role of Phenolics, Polysaccharides, Alcohol, and pH in White Wine Structure and Style

**Richard Gawel**,* Martin Day, Alex Schulkin, Steve van Sluyter, Elizabeth J. Waters, and Paul A. Smith
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White wines that complement food are often characterized by a firm palate structure, which is often attributed to phenolics and polysaccharides extracted during must handling. The tastes and textures of the two most abundant phenolics in white wines, grape reaction product (GRP) and caftaric acid (indicative of oxidative and low oxygen juice handling, respectively), were quantified. Caftaric acid reduced the astringency and hotness directly resulting from lower pH and higher alcohol levels, and GRP produced an increase in oily texture. Wine polysaccharides had little effect on wine fullness, but reduced alcohol hotness. We also describe the effect of conventional and less-used winemaking practices such as whole bunch pressing, skin contact, pressings, hyperoxidation, solids, and partial skin fermentation on the phenolic, polysaccharide, and sensory profiles of Chardonnay, Riesling, and Viognier wines over three vintages.

*Funding support:* Australian Grape and Wine Research and Development Corporation and Orlando Wines

### Influence of Alcohol on the Sensory Properties of Cabernet Sauvignon Wines

**Ellena S. King**, Randy Dunn, and Hildegarde Heymann*
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Over the last 20 years, alcohol concentrations in New World wines have increased dramatically. The causes of this increase include changes in climate and wine styles. The aim of this project was to assess how alcohol concentration influences Cabernet Sauvignon wine sensory profiles. Twenty-four predominantly Cabernet Sauvignon, commercial U.S. wines were selected to encompass a broad range of wine styles and alcohol concentrations between 12 and 16% v/v. Low- and high-alcohol wines were defined as below or above 14% v/v, respectively. Three descriptive sensory analyses were conducted by 11 to 12 trained panelists each: a Random group assessed the wines in a random order; a HiLo group assessed the high-alcohol wines before the low-alcohol wines, and a LoHi group assessed the low-alcohol wines first. From an ANCOVA, using measured alcohol concentration as covariate, alcohol positively influenced the perception of alcohol and burning, as expected, in

**Enology — Sensory Session – CONTINUED**

addition to chemical aroma, bitterness, viscosity, sharpness, flavor length, flavor intensity, and complexity. Alcohol negatively affected fresh fruit and berry aromas and smoothness. These results indicate that high-alcohol concentrations can enhance some undesirable sensory descriptors. The order of wine assessment significantly affected sensory perception. The LoHi group was the most discriminating based on alcohol concentration. This group perceived mostly positive correlations between alcohol concentrations and sensory attributes, while the HiLo group identified more inverse relationships. The Random group exhibited more varied and intermediate responses. Similar results were obtained from a wine show judging panel using the same order effects. The results indicate that initial assessment of high-alcohol wines can reduce perception of aroma and taste descriptors, while the opposite is true when low-alcohol wines are assessed first. This suggests the need to consider wine alcohol concentrations when professionally assessing wine quality, such as at wine shows.

*Funding support:* University of California Davis, George Murray Scholarship, University of Adelaide, Beckstoffer Vineyards, Bob Egelhoff, Charles Krug, Peter Mondavi Family Winery, Cone Tech, Inc., Cathy Corison, Corison Winery, Cornerstone Cellars, Dunn Vineyards, Frog's Leap Winery, Miner Family Winery, Raymond Vineyards and Cellars, Silver Oak Cellars, and Woodward Canyon Winery

## Characterization of Key Odorants in Central Otago Pinot noir Wines

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The aroma of Pinot noir wines from the Central Otago region of New Zealand was examined using aroma extraction dilution analysis (AEDA), headspace analysis via purge and trap, quantitative analysis of the main odorants, and aroma reconstitution and omission tests. Two representative wines were selected for analysis over three consecutive vintages. Forty compounds were identified in the AEDA study, with flavor dilution factors ranging from 3 to 19,683, and over 20 compounds had flavor dilution factors >81. Over 18 odorants were detected using gas chromatography/olfactory after purging the headspace from selected wine samples and trapping onto Lichrolut EN resin. Quantitative analysis of over 60 volatiles was achieved using liquid:liquid and solid-phase microextractions followed by GC-MS analysis. The odor-active families of compounds with potential importance included ethyl esters of fatty acids, higher alcohol acetates, cinnamic esters, terpenols,

$C_{13}$-norisoprenoids, β-phenyl ethyl alcohol, isobutyric and isovaleric acids, benzaldehyde, and several oak-derived volatiles. Eighteen aroma models were prepared by deodorizing two Pinot noir wines with Lichrolut EN resin and reconstituting with purified aromatic compounds. A control model was reconstituted up to the original concentrations of the major compounds, while the other models were prepared with the omission of an aroma family or particular compound. The models were presented to a trained panel asked to evaluate the intensity of the differences of the new models from the original wine using an agreed set of descriptors. Those compounds found to make a profound sensory difference through omission were considered key odorants in Central Otago Pinot noir wines.

*Funding support:* New Zealand Winegrowers

## Effect of Storage Temperature and Packaging Type on the Sensory Properties of Chardonnay

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The shelf life of wine after bottling or filling is a crucial aspect of the final quality seen by the consumer. Yet, in most instances, shelf life is outside the control of the winemaker. Factors like storage temperature and packaging type are important in determining wine shelf life. California Chardonnay (vintage 2010) was stored at three different constant temperatures (10, 20, and 40°C) for three months in five different packaging configurations: 0.75-L glass bottles with ROTE screwcaps, natural corks, or synthetic corks and 3-L bag-in-boxes with and without modified atmosphere packaging during filling. During storage, changes in headspace and dissolved oxygen were monitored using noninvasive, luminescence-based sensors. After storage, all samples were analyzed using a generic descriptive analysis for aroma, flavor, taste, and color by 14 trained panelists. Significant differences among wine samples were found by the sensory panel for nine aroma, three taste and mouthfeel, and three color attributes; the two bag-in-box treatments showed particularly dramatic changes. Statistical analysis of the oxygen data collected during the storage showed very large and significant differences in oxygen consumption among the different packaging configurations and storage temperatures that correlated well with the descriptive analysis findings.

*Funding support:* Jerry Lohr and Constellation Wines US

## Enology — Sensory Session – CONTINUED

### Evaluating the Effects of Membrane Filtration on Sensory and Chemical Properties of Wine

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It is a long-held belief in the wine industry that membrane filtration, specifically sterile filtration below 0.45 µm, will strip a wine of aroma and color. For this reason, many winemakers avoid the use of sterile filters in wine production, which can cause uncertainty in microbial stability of the finished wine. There are currently no studies connecting sterile filtration to a significant sensory effect on finished wine. To assess this, two red wines, a Cabernet Sauvignon and a Merlot, and one aromatic white wine blend were filtered through 0.45-µm polyethersulfone (PES) and polyvinyl difluoride (PVDF) membrane filters. Sensory and chemical characteristics of these wines were compared to unfiltered control wines. Treatments were expanded with the Merlot and white wine blend to also examine the effects of a pad filter and a cartridge depth filter used as prefilters. Possible changes in dissolved oxygen were monitored during bottling, while tannin concentration and color were examined through the course of filtration. There were minor differences in tannin and color after pad filtration, but there was no significant variation during the course of the filtration. Descriptive sensory analyses were conducted for each wine immediately following filtration and on a regular basis for up to 24 weeks. While all three wines exhibited significant variation in sensory descriptors over time, a decrease in astringency between control and filtered Merlot wines was the only significant variation among treatments. Overall, there was no significant impact of sterile filtration on the sensory or chemical properties of the wines tested, regardless of the type of filter material used.

*Funding support:* American Vineyard Foundation

## Viticulture — Pests and Diseases

### Map-based Cloning of Pierce's Disease and *Xiphinema index* Resistance Genes from *Vitis arizonica*

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Map-based or positional cloning is an experimental approach that enables identification of genes associated with important agronomic traits. This process requires construction of a high-density genetic map to correctly position a gene of interest between closely flanking markers. The Walker lab has developed several high-density maps that have been used to enable marker-assisted selection and expedite the breeding of rootstock and scion cultivars. Two independent genetic mapping projects using resistance from *Vitis arizonica* have resulted in the localization of:
(1) a homozygous dominant locus, designated *PdR1*, responsible for Pierce's disease (PD) resistance; and (2) a major quantitative trait locus (QTL), designated *XiR1,* which confers resistance to the nematode *Xiphinema index*. Physical mapping and sequence analysis around both loci resulted in the identification of two and six candidate genes for *XiR1* and *PdR1b,* respectively. The final step to confirm the function of these genes is their transformation into susceptible *Vitis* cultivars via *Agrobacterium tumefaciens*. Because tobacco is very easy to transform and susceptible to PD and tomato is also easy to transform and susceptible to *X. index*, we have also transformed these species. We now have plants of Thompson Seedless and *V. rupestris* St. George transformed with *XiR1.1* and *XiR1.2* and plants of tobacco transformed with *PdR1b.1* and *PdR1b.6*. Preliminary results showing the response of these transformed plants against *X. index* and *Xylella fastidiosa*, the causal agent of PD, will be presented.

*Funding support:* CDFA PD/GWSS Board, California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, CDFA Improvement Advisory Board, California Table Grape Commission, and Louis P. Martini Endowed Chair for Viticulture

### Effects of Grapevine Leafroll Disease on Own-Rooted Winegrape Cultivar Merlot under Cool-Climate Conditions

**Olufemi J. Alabi**, Linga R. Gutha, Luis F. Casassa, James Harbertson, Maria Mireles, Christopher W. Beaver, Joan Davenport, and Rayapati A. Naidu*
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We conducted a three-year study on effects of grapevine leafroll disease

## Viticulture — Pests and Diseases – CONTINUED

(GLRD) on performance and fruit quality of a winegrape cultivar (Merlot) planted as own-rooted vines under cool-climate conditions in Washington State. Vines exhibiting GLRD symptoms (tested positive for Grapevine leafroll-associated virus-3) and healthy vines, planted adjacent to each other in a given row in a commercial block, were selected to minimize error in experimental results due to variations in growing conditions. Analysis of macro- and micronutrients in leaf samples collected from GLRD-affected and healthy vines at midbloom and late veraison excluded the possibility of nutrient deficiency as factor in GLRD symptoms. Juice from berries collected randomly from each vine at different berry development stages were analyzed for fruit maturity indices (soluble solids and fruit acidity) and total anthocyanins throughout the season until harvest. Results from three seasons clearly indicated negative impacts of GLRD on fruit maturity indices, with differences between grapes from healthy and diseased vines widening as berry maturation advanced. Cumulative fresh fruit yield per vine at commercial harvest was significantly reduced in GLRD-affected grapevines. Small-lot wine made from grapes harvested from GLRD-affected vines had significantly lower alcohol, polymeric pigments, anthocyanins, total iron-reactive phenolics, and tannins than wine made with grapes from healthy vines. The observed negative impacts of GLRD on vine performance and fruit and wine quality were consistent in all three seasons, although the magnitude of the impacts differed among seasons. Descriptive analysis of small-lot wines indicated that wines from healthy vines were more purple and more saturated in color, with a predominance of red fruit aromas and less earthy aromas. Results clearly showed negative impacts of GLRD on vine performance and fruit and wine quality of own-rooted Merlot under cool-climate conditions of Washington State.

*Funding support:* Agriculture Research Center, Washington State University College of Agriculture, Human and Natural Resource Sciences, Wine Advisory Committee of the Washington Wine Commission, and USDA-National Institute of Food and Agriculture-Specialty Crop Research Initiative Award 2009-51181-06027

### Genetic Analysis of Foliar Phylloxera in Northern California

**Karl Lund**, Summaira Riaz, and M. Andrew Walker*
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Grape phylloxera feed abundantly on the leaves of grapes in their native range; however, foliar feeding is very unusual in California vineyards and nurseries. Over the past five years, grape rootstock nursery plantings in Yolo and Solano counties have been infested by the foliar phase of grape

phylloxera. This outbreak has spread to the National Clonal Germplasm Repository in Solano County and the Foundation Plant Services vineyards in Davis. To determine the source or sources of this infestation, phylloxera were collected from both the leaves and roots of infested rootstocks for use in a genetic diversity study. Phylloxera were also collected from roots in northern California vineyards for comparison with the foliar forms. The samples were tested with SSR markers previously used in genetic diversity studies in Australia, Europe, and California and with a new set of SSR markers recently developed by the Walker lab. The foliar phylloxera strains had very limited genetic diversity. This suggests that the foliar phylloxera currently present in Yolo and Solano counties are the result of a single introduction that has spread across rootstock nursery plantings and vineyards in both counties. This study also demonstrated that the newly developed SSR markers are more efficient and highly polymorphic.

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

## Complete Cluster Zone Leaf Removal Conducted Early in Berry Development Helps Reduce Disease and Increase Fruit Composition during Cool Seasons in Oregon

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Partial cluster zone leaf removal is typically conducted after fruit set to increase exposure for fruit development. A multiyear study was conducted to compare different timings of complete cluster zone leaf removal in Pinot noir. Leaf removal at early berry developmental stages from prebloom to post-fruit set may be beneficial in increasing fungicide penetration and altering microclimate to enhance disease control and berry development. Leaf pulling was conducted by hand at five different stages: flower separation, 50% bloom, fruit set, pea-sized berries, and bunch close. All treatments removed between five to seven basal leaves per shoot, exposing inflorescences or clusters completely, and treatments were kept free of leaves in the cluster zone from treatment initiation. All were compared to an unpulled treatment. The 2008 to 2011 season data indicate that early season leaf pulling decreased disease severity of grape powdery mildew and *Botrytis*. Leaf removal prior to bunch closure reduced disease incidence and severity more than foregoing pulling entirely. The earliest leaf pulling conducted prior to bloom reduced fruit set by 20% in the two cool seasons. Early season leaf pulling

**Viticulture — Pests and Diseases – CONTINUED**

reduced berries per cluster and cluster weight, but there were no differences in berry weight in any year of the study. Differences in berries per cluster may have helped to reduce disease infection. Leaf pulling did not delay fruit ripening or impact soluble solids, pH, and tritatable acidity by harvest; however, there were increased total anthocyanins, phenolics, and tannins in 2010 and 2011 in the earliest leaf pull treatments compared to bunch close leaf pull or unpulled treatments. Sunburned fruit was not observed in any year, despite complete exposure of clusters.This work indicates that complete cluster zone leaf pulling is a viable management option for vineyard IPM.

*Funding support:* Oregon Wine Board and Viticulture Consortium-West

### Effects of Preflowering Defoliation on Cluster Architecture, Rot Incidence, and Grape Composition

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Canopy management is an important tool for viticulturists. Leaf removal to alter the cluster microclimate can improve fruit quality and reduce bunch rot. However, excessive exposure in warm climates can lead to sunburn and reduce fruit quality. The timing of leaf removal is important, as removal of foliage changes the source to sink ratio and can affect ripening in the current and following season. A technique gaining interest recently is the removal of leaves prior to flowering. Early defoliation leads to setting fewer berries per bunch. Looser bunches are less prone to rot and when a fungal infection occurs, rots spread more slowly in looser bunches than in tight ones. The reduced yield from early defoliation can also be beneficial for grape composition, especially in regions that do not have the luxury of extended hangtime and therefore regularly practice fruit thinning. We compared Chardonnay, Sauvignon blanc, Pinot gris, and Merlot vines defoliated prior to flowering to those defoliated at prebunch closure (the standard in New Zealand). In general, early defoliated vines had fewer berries per bunch, looser bunches, reduced yield, and reduced rot severity and incidence. There were very few differences in grape composition between treatments, in contrast to many previous studies on early defoliation. There were no significant differences in wine sensory properties as measured using the Napping technique. Early defoliation is a viable technique to limit crop and reduce bunch rot, both important considerations in a cool-climate growing region such as New Zealand.

*Funding support:* New Zealand Winegrowers, Hawke's Bay Winegrowers, and Ministry of Agriculture and Forestry Sustainable Farming Fund

## Enology — Wine Flavor Session

### Prevalence and Impact of Dimethyl Methoxypyrazine in Wine

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Alkyl-methoxypyrazines play an important role in the aroma and flavor of some wines. As endogenous components of grapes, they contribute to the typicity of some varietal wines, including Sauvignon blanc and Cabernet Sauvigon. However, they can also be derived from Coccinellidae species (*Coccinella septempunctata* and *Harmonia axyridis*), where they are responsible for producing ladybug taint in wine. Four methoxypyrazines have been identified in Coccinellidae haemolymph: isopropyl methoxypyrazine, isobutyl methoxypyrazine, secbutyl methoxypyrazine, and dimethyl methoxypyrazine. Of these, isopropyl methoxypyrazine, isobutyl methoxypyrazine, and secbutyl methoxypyrazine have also been identified in several grape varieties and their wines. In this study, we sought to determine the prevalence and significance of dimethyl methoxypyrazine in a range of untainted wines. The dimethyl methoxypyrazine concentrations of 250 samples of seven varietal wines from around the world (Cabernet Sauvignon, Cabernet franc, Merlot, Pinot noir, Sauvignon blanc, Riesling, and Chardonnay) were determined using solid-phase microextraction followed by multidimensional gas chromatography–mass spectrometry (SPME-MDGC–MS). Additionally, gas chromatography mass spectrometry coupled with olfactometry (GC-MS/O) was used to assess the odor of dimethyl methoxypyrazine in three white and three red wines. GC-MS/O data showed that the odor of dimethyl methoxypyrazine in wine can be described as earthy, musty, vegetal, and dead leaves/flowers. Further, the presence of dimethyl methoxypyrazine in wine not affected by ladybug taint is reported for the first time. Its source, concentration, and prevalence are discussed in the context of its sensory threshold and purported impact on wine quality.

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## Enology — Wine Flavor Session – CONTINUED

### Profiling Toasting Levels of Oak Stave Wood in New Wine Barrels Using UHPLC/QTOF-MS

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Oak barrels for wine storage are toasted over wood fires during the barrel-making process. The toasting process produces a number of volatile compounds from the lignin and cellulose components of the wood and a range of polyphenols from the lignin and ellagitannins. This study used UHPLC/QTOF-MS to profile nonvolatile compounds in extracts of wood collected at different positions along the length of barrel staves. The objective was to understand how variations in temperature of the wood that occur during toasting might affect the nonvolatile profile of the toasted wood. Staves from five barrels made from a single wood lot were selected and toasted using a standard protocol. Samples from the wood were extracted in a model wine solution. The extracts were analyzed using an Agilent 1290 UHPLC/6430 QTOF MS system. Separation was accomplished on a 5 cm x 1.8 µm Zorbax Eclipse plus C18 column with an acetic acid/methanol-acetic acid gradient. The QTOF-MS was run in negative mode with a mass range of 100 to 1700 *m/z*. Total analysis time was approximately 12 min per sample; each wine was analyzed in triplicate. Data analysis was conducted using MassHunter Qualitative Analysis and Mass Profiler Professional software. Analysis of the MS data using multivariate statistics produced a set of accurate masses and retention times that could be used to discriminate staves in this set. This approach was able to differentiate samples from the different staves in the set and to a lesser extent was able to differentiate samples taken from different locations along each of the individual staves.

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**Enology — Wine Flavor Session – CONTINUED**

## Ethanol, Tannin, and Fructose Interaction Effects on the Headspace Concentration of Odorants and Their Potential Contribution to the Aroma of Model Wine

**Remedios R. Villamor**,* D. Scott Mattinson, Marc A. Evans, and Carolyn F. Ross
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The objective of this study was to investigate the effects of ethanol (8, 10, 12, 14, and 16%), tannin (500, 1000, and 1500 mg/L grape tannin, Biotan), and fructose (200 or 2000 mg/L) concentrations on the headspace concentration of eight selected odorants and explore their potential contribution to the aroma of a model wine. The aroma compounds were extracted using headspace solid-phase microextraction and analyzed using gas chromatography-mass spectrometry. There were significant three-way interaction effects for most of the aroma compounds ($p < 0.05$). In general, tannin exhibited salting-out effects while fructose induced salting-in, both of which were largely dependent upon ethanol. The net magnitude effect was a substantial reduction in the headspace concentration of odorants, with the dominant contribution from ethanol. The percent reduction in odorant recovery was greater for the larger compounds: 1-octen-3-one (72.4%), 2-methoxyphenol (74.3%), 2-phenylethanol (74.9%), eugenol (70.7%), and β-damascenone (75.5%). Decrease in volatile recovery was less pronounced for low molecular weight compounds 3-methyl-1-butanol (57.2%), dimethyl disulfide (58.0%), and 1-hexanol (61.7%). In a subsequent experiment, gas chromatography/olfactometry analysis revealed differences in the estimated odor thresholds of odorants in model solutions with varying ethanol (10 or 14%), tannin (500 or 1500 mg/L grape tannin, Biotan), and fructose (200 or 2000 mg/L). Threshold values increased between two- and 10000-fold for 2-methoxyphenol and eugenol, respectively, at higher ethanol, tannin, and fructose concentrations. Consequently, higher tannin and fructose concentrations at 10% or 14% ethanol decreased odor unit values, indicating a reduction in the potential contribution of the odorants to the aroma of model wine. These results highlighted the significant impact that wine matrix interactions can have on wine aroma quality.

*Funding support:* Washington State Grape and Wine Research Commission and the Northwest Center for Small Fruits Research

## Enology — Wine Flavor Session – CONTINUED

### Dry versus Soaked Wood: Modulating the Volatile Extractible Fraction of Oak Wood by Heat Treatments

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Initially intended as a wine-suitable container, oak barrels are now considered to have a major effect on wine. Sensory properties acquired by wine during barrel aging depend on many different factors, including the initial wine composition, aging duration, and wood composition; the latter is most critical for aromatic and/or tannic impact. Wood composition is highly affected by heat treatments during barrelmaking, where the first aim is to bend the staves and the second critical aim is to finetune the concentration of volatile compounds through controlled degradation of macromolecules. Here we show that the initial water content of cooperage oak wood can have a major impact on the efficiency and yield of the heating process, leading to unexpected concentrations of important aromatic compounds such as *cis*- and *trans*-whiskey lactone, vanillin, guaiacol, furfural, and eugenol, which can be extracted by a model wine. Such results, for instance, higher concentrations of whiskey lactones after high-temperature treatments of soaked wood instead of dry wood, cannot be explained simply by a delaying effect of the water content on the heat transfer rate. Modifications of the lignocellulosic macromolecular network as a result of water adsorption have to be considered. Most importantly, the observation of two different temperature-related behaviors as a function of heating durations of soaked wood clearly indicate that the high water content of such wood leads to a significantly different structural modification of the macromolecular network upon heating, and that the potential for wood-related aromatic extraction is thus significantly modified. Solid-state NMR analyses further support our hypotheses.

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## Viticulture — General Session

### Balancing the Perspectives on Vine Balance

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Experiments were conducted over multiple years in several major wine-growing regions of California to examine the impact of vine balance metrics (defined either as the ratio of vine leaf area to fruit weight or the ratio of fruit yield to pruning weight) on the development of impact aroma and mouthfeel compounds in Cabernet Sauvignon grapes and wines. Grapevines were cluster-thinned immediately prior to veraison to create a wide variety of crop load treatments, ranging from significantly overcropped (yield:pruning weight ratio >12) to undercropped (yield:pruning weight ratio <4). In addition, other key vine performance metrics were measured, including the percentage of total vine leaf area exposed to sunlight at midday and leaf layer number in the fruiting zone. As expected, undercropped vines accumulated sugar more rapidly than balanced (optimum cropped) or overcropped vines. However, the accumulation of impact aroma compounds was uncoupled from sugar accumulation; balance cropped vines accumulated dark fruit aromas (β-damascenone) more rapidly and had lower levels of green aroma compounds (isobutyl methoxypyrazine) than the other treatments. A similar uncoupling with sugar accumulation was observed for berry color and mouthfeel compounds. As a result, optimum flavor profiles in both grapes and wines were reached at lower soluble solids levels in balanced cropped vines compared to vines that had been excessively cluster thinned and undercropped. The results of this study illustrate the importance of crop load in regulating fruit flavor development during ripening as well as the potential to obtain optimum flavors at lower soluble solids levels when crop loads are properly regulated.

*Funding support:* E&J Gallo Winery

### Determining the Impacts of Crop Thinning on Vine Vigor and Fruit Composition in Oregon Pinot noir

**Amanda J. Vance** and Patricia A. Skinkis*
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Managing crop levels is a common practice in cool-climate vineyard production to achieve vine balance and desired fruit quality, although it is costly. With economic pressures, vineyard managers are questioning whether they can reduce production costs and increase yields without compromising quality. A

**Viticulture — General Session – CONTINUED**

crop thinning trial was conducted in 2010 and 2011 to address these concerns and to better understand the role of vine balance on fruit composition in Pinot noir. Crop levels were moderately (35% crop removed) or severely (65% removed) thinned at prebloom, fruit set, lag phase, or veraison and compared to unthinned vines. In both years, crop thinning reduced yields without causing berry size compensation. In 2010, poor fruit set reduced overall yields and thinning treatments resulted in very few differences in vine growth, cluster architecture, or fruit composition, including total soluble solids (TSS), pH, titratable acidity (TA), yeast assimilable nitrogen (YAN), anthocyanins, phenolics, and tannins. In 2011, yields were much higher due to high fruit set and resulting cluster size. No differences were found in vine growth (leaf area or pruning weights) or fruit YAN, but thinned vines had higher TSS and pH and lower TA than unthinned vines. Fruit thinned at lag phase and veraison had higher TSS and lower TA than fruit thinned prebloom. Intensity of thinning had a stronger influence on anthocyanin and tannin concentration than timing, while phenolics were not impacted by either factor. Ravaz indices (fruit yield/pruning weight) below 2.25 and leaf area to yield ratios of 2.25 to 3.25 $m^2$/kg improved fruit composition in 2011, although data from the remaining years of this study will provide more insight into appropriate crop load metrics for cool-climate Pinot noir.

*Funding support:* the Northwest Center for Small Fruits and the Oregon Agricultural Research Foundation

## Irrigation Frequency and Rate Effects on Vine Growth, Leaf Gas Exchange, Berry Composition, and Wine Sensory Quality in Red Winegrapes

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The effects on Merlot, Cabernet Sauvignon, and Syrah of irrigating at two deficit rates and two frequencies (daily and every three days) were determined over four years in field experiments conducted in commercial vineyards on loamy sands. Daily irrigation resulted in shallower moisture penetration in the soil profile and less variable soil moisture levels over irrigation cycles. In all varieties, vine growth, as indicated by pruning mass, was reduced in vines irrigated at the lower rate but was unaffected by irrigation frequency. Leaf gas exchange rates were higher, especially after veraison, in response to irrigating daily or at the higher rate. Irrigation effects on yield were inconsistent among cultivars and years, but in the year when yields

were highest in all varieties, daily irrigation produced lower yields. Juice pH was lower in response to irrigating daily or at the higher rate. Irrigating daily or at the lower rate produced berries with lower mass in all varieties and with higher soluble solids and anthocyanin concentrations in Merlot and Syrah. Skin and seed tannin contents of berries were higher in all varieties when irrigated daily but were affected little and inconsistently by irrigation rate. In the final two years of the study, irrigation frequency effects on Merlot wine sensory characteristics were evaluated. In response to daily irrigation, the wines had more body and more intense color in both years, and in one year they had less intense herbaceous aroma, more intense spicy aroma, and black and red fruit aromas and flavors, and greater longevity on the palate.

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

## Evaluation of 55 Winegrape Cultivars for the San Joaquin Valley

**Matthew W. Fidelibus**, James A. Wolpert,* Kimberley A. Cathline, and Stephen J. Vasquez
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The first trial, evaluating 20 red wine cultivars, concluded in 2010 and was reported at the 2011 ASEV National Conference in Monterey, CA. The current experiment investigated the growth, yield, and fruit composition of 55 cultivars, 27 for white wine and 28 for red wine. The cultivars originated primarily in France, Italy, Spain, and Portugal and budwood was sourced from either Foundation Plant Services at UC Davis or nurseries that hold proprietary rights. The experiment was conducted at the Kearney Agricultural Research and Extension Center. The vineyard was planted on a Hanford Fine Sandy Loam soil, at a spacing of 1.8 m x 3.0 m (6 ft x 10 ft, vine by row) on 1103 Paulsen rootstock (*V. rupestris* x *V. berlandieri*), trained to a bilateral cordon, and pruned to 30 buds per meter. The experimental design was single, unreplicated plots of 12 to 15 vines per cultivar, a screening trial. White cultivars were harvested at 22 Brix and red cultivars at 24 Brix. In 2011, white cultivars that reached the target Brix were harvested from as early as 23 Aug to as late as 17 Oct. Red wine cultivars that reached the target Brix were harvested from as early as 24 Aug to as late as 3 Nov. Five white cultivars and eight red cultivars did not reach the target Brix despite delaying harvest until early November. Dramatic differences were seen in growth, yield components, rot levels, and fruit composition for both white and red cultivars. Correlations among the measured components and strategies for 2012 will be discussed.

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Table of Contents List of Authors *indicates corresponding author

## Viticulture — Water Relations Session

### Timing and Severity of Regulated Deficit Irrigation on Cabernet Sauvignon. Grapes: Agronomical and Compositional Effects

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A 30-year-old Cabernet Sauvignon vineyard located in Mattawa, WA, was subjected to four alternative regulated deficit irrigation (RDI) treatments: (1) 100% reposition of evapotranspirative demand (ETc); (2) 70% ETc; (3) 25% ETc until veraison, followed by 100% ETc until harvest (25/100%); and (4) 25% ETc. Yield per vine was low in 25% ETc and 70% ETc and ~40% higher in 100% ETc and 25/100% ETc. Midday stem water potential followed the pattern 100% ETc > 70% ETc > 25/100% ETc > 25% ETc. Berry weight was ~31% in 25% ETc. At harvest, no differences in Brix and only minor differences in TA and pH were observed. The contribution of malvidin derivatives to the total anthocyanin profile increased progressively from veraison (~50%) to harvest (~70%). To disentangle a possible "concentration" effect (i.e., smaller berries) from a biosynthetic effect, several phenolic families were analyzed separately in both skin and seeds, and results expressed both on a fresh weight and per berry basis. On a fresh weight basis, 25% ETc and 25/100% ETc resulted in higher concentrations of skin tannins, total anthocyanins, and total flavonols, but no clear trend was observed for seed tannins. On a per berry basis (i.e., biosynthesis effect), berry weight was negated and results showed that RDI decreased seed tannins. On skins, 25/100% ETc resulted in higher concentrations of skin tannins but malvidin derivatives were higher in 25% ETc and 25/100% ETc. As previously observed, in long, cool vintages, color and tannins are biosynthetically less affected by irrigation treatments. From a practical standpoint, 25/100% Etc appears to be a viable irrigation strategy to control vegetative vigor and berry size and enhance the phenolic quality of skins, while maintaining yield/vine and overall vegetative/reproductive performance.

*Funding support:* Wine Advisory Committee, Washington Wine Commission, and WSU Agricultural Research Center, Fulbright Commission, Walter Clore Scholarship, Ministerio de Educacion (Programa Jose Castillejo), and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria project RTA2008-00037-C04-04

**Viticulture — Water Relations Session – CONTINUED**

## Fruit Uniformity in Winegrape Production: Relation to Water Deficits, Cluster Thinning, and Crop Value in Commercial Vineyards

**Arturo Calderon-Orellana**, Kenneth Shackel, William Drayton, and Mark Matthews*
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Although fruit uniformity is widely considered as a key quality parameter in winegrape production, no study has specifically addressed two fundamental questions: Is fruit uniformity related to wine quality and can cultural practices improve fruit uniformity? Two experiments were carried out in commercial Cabernet Sauvignon vineyards in California to evaluate the impact of viticultural management on fruit uniformity. In Experiment 1, three crop value levels ($/ton), presumably reflecting substantial differences in fruit quality, were selected as high ($9,000–10,000), medium ($4,000–5,000), and low ($500–1,000), from different locations in the North and Central Coast wine regions of California for two consecutive years. Random cluster samples were picked at harvest for each vineyard and fruit uniformity was evaluated at different levels (vine-to-vine, cluster-to-cluster, etc.) for Brix, pH, berry weight, and anthocyanin concentration. In Experiment 2, irrigation and cluster thinning treatments were imposed in a commercial vineyard in the Dunnigan Hills (CA) region for three consecutive years. Measures of fruit uniformity included a variance components analysis and an ANOVA on absolute deviations (residuals) for Brix, pH, anthocyanins, and berry weight. Despite differences in location and management, uniformity of ripeness, fruit color, and berry size were not related to crop value. Clusters from high value sites showed numerically lower uniformity for most fruit parameters measured. Postveraison water deficits significantly reduced fruit uniformity due to fruit dehydration. Higher berry temperature and greater light penetration were also observed in vines under water deficits. Fruit uniformity was significantly improved by cluster thinning at veraison, but was not affected at harvest. Differences in the effect of cluster thinning on fruit uniformity between veraison and harvest were attributable to changes in fruit uniformity over fruit development.

*Funding support:* Fulbright Commission, Ministry of Education of Chile, University of California, Davis, Chilean Wine Consortium (TECNOVID), University of Chile, ASEV, Constellation Wines U.S., and Treasury Wine Estates

## Viticulture — Water Relations Session – CONTINUED

### Testing the Theory of Hydraulic Autonomy of Grapevine Shoots under Water Stress

**Vinay Pagay**,* Vivian Zufferey, and Alan N. Lakso
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This study attempted to determine whether shoots of varying vigor within individual grapevines (*Vitis vinifera* L.) under water stress respond differently hydraulically, that is, whether shoots regulate their water use independently of other shoots on the same vine. Based on our initial observation that shoots of varying vigor had different stem water potentials early in the season, we tested the hypothesis that high-vigor shoots (HV; shoot length >2.0 m) have stricter regulation of water use than low-vigor shoots (LV; shoot length <1.2 m) to avoid hydraulically disruptive xylem cavitations, thereby maximizing shoot growth and productivity. Using a combination of leaf gas exchange, acoustic emissions, and high pressure hydraulic measurement techniques during the 2011 growing season on field-grown Riesling grapevines in the Finger Lakes region of New York, we found that: (1) HV shoots were more sensitive to water stress than LV shoots; (2) LV shoots had a lower cavitation threshold or greater cavitation vulnerability than HV shoots; and (3) HV shoots showed near isohydric behavior while LV shoots were relatively anisohydric. These findings provide support to our theory of at least partial shoot hydraulic autonomy under water stress and could have implications for canopy management practices in viticulture.

*Funding support:* Cornell Horticulture

### Evaluation of the Impacts of In-Row Vineyard Floor Management Practices on Soil and Water Erosion, Vine Growth, and Productivity of Grapevines

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One large-plot and one small-plot experiment were established in 2005 in a first leaf vineyard to evaluate the impact of in-row barley cover cropping on soil and water erosion, vine growth and productivity, weed control, and soil characteristics. A 1.4-hectare experimental site evaluated three floor management practices (in-row treatments): (1) standard, preemergent herbicide using flumioxazin (Chateau) at 0.43 kg a.i./ha + 2% glyphosate, with follow-up postemergence applications of 2% glyphosate applied as needed in the summer to control escaped weeds; row middles had a barley cover crop; (2) standard + bare middles; and (3) in-row cover crop using barley that developed to 30 cm and then burned back with a 2% glyphosate

application + 0.43 kg a.i./ha flumioxazin; row middles had a barley cover crop. A small-plot experiment was also established to evaluate the influence of timing of burn-back herbicide sprays to in-row covers. Results from 2006 to 2010 showed cover crops reduced soil and water erosion from winter rains and the nutrient content of runoff water compared to bare soil. There was no significant difference in erosion with the additional in-row cover as compared to cover in the row middles only under the low rainfall common to the trial site. In-row covers chemically killed at 30 cm had no negative influence on growth or productivity. In-row covers that were allowed to grow just prior to budbreak showed a trend toward reduced shoot growth but did not reduce crop yield. In-row covers increased soil organic matter and microbial activity. Plant tissue at bloom had more potassium and phosphorous and less nitrogen with in-row cover cropping. Soil moisture levels were lowest in the in-row cover treatment during March and April, indicating higher water use; however, during the summer, soil moisture levels were highest in the in-row treatment, indicating a change in the soil to influence water movement.

*Funding support:* American Vineyard Foundation, Viticulture Consortium West, Monterey County Vintners and Growers Association, and Valley Farm Management

## Continued Screening for Chloride Exclusion in Wild Grapevines: New Collections and Genetic Information

**Claire Heinitz** and M. Andrew Walker*
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Wild *Vitis* species from the southwestern United States present an excellent resource for breeding salt-tolerant rootstocks. Previous greenhouse screens have shown that chloride exclusion is widespread among genotypes from this region, although the taxonomic and geographic origins of the trait are still unclear. We have since expanded the screen to include a more intensive study of regions of interest and have obtained many new accessions by collecting in southern Arizona and New Mexico. Expanded chloride exclusion results will be presented, along with an analysis of a screening technique using intact, rooted leaves of *V. girdiana* and *V. arizonica*. The rooted leaf technique, which involves trimming the leaf and forcing roots from the base of the petiole, provides an interesting system for studying variation in chloride uptake across different species. Finally, results from a study of the genetic diversity among southwestern U.S. *Vitis* using microsatellite markers will be presented.

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

Table of Contents List of Authors *indicates corresponding author

## Enology — Wine Stability and Oxidation Session

### Recent Progress on Heat Stability in White Wines

**Liz Waters*** (Keynote Speaker)

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Haze and turbidity in white wines is visually unattractive and thus unacceptable to the consumer. One of the major types of haze in white wine is protein haze. The proteins that cause haze occur naturally in the grape, and, because they are robust, survive fermentation and most winemaking practices. Unless they are removed by absorption onto bentonite, they remain soluble in the finished wine. Over time after bottling, the proteins can aggregate together into large enough particles to scatter light and thus be visible to the naked eye. This presentation will include an overview of our current understanding of why and how protein haze forms and the wine components other than protein that are involved; viticultural and winemaking practices that influence the levels of these proteins in wine and thus the potential of a wine to produce haze; practical solutions to improving bentonite efficacy to remove proteins and thus prevent protein haze formation; an update on research into alternatives to bentonite.

### Recent Progress in Cold Stability in Wines

**Roger B. Boulton*** (Keynote Speaker)

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The treatment of wines in order to prevent the precipitation of potassium bitartrate during low temperature exposure associated with post-bottling shipping and handling continues to be an issue with various alternative solutions. Recent progress in treatment technology ranges from the development of fluidized-bed crystallizers to electro-dialysis and the addition of nucleation inhibitors to prevent crystal formation. These solutions have advantages and disadvantages associated with them and various intensities of energy and water consumption or corresponding polymer residuals. There has been a related growth in lab automation of a number of stability tests and these have varying degrees of confidence when applied to winery treatments. The treatment requirements, potential for tartrate recovery, loss of mineral fingerprint, water and energy consumption are compared quantitatively and the concerns of the inhibitor addition approach are addressed. Future directions for process developments and scientific understanding are presented.

## Quinone Reaction Kinetics Suggest Oxidation Management Strategies

Maria Nikolantonaki and **Andrew L. Waterhouse***
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Quinones are reactive electrophilic oxidation intermediates in wine, so 4-methyl-1,2-benzoquinone was used as a model compound to study reactions with wine nucleophiles. The Michael addition reactions of the quinone addressed varietal volatile thiols, hydrogen sulfide, glutathione, sulfur dioxide, ascorbic acid, phloroglucinol, and the amino acids methionine and phenylalanine in the first kinetic study of these reactions. These produced adducts in fairly good to quantitative yields. The reaction rates of 4-methyl-1,2-benzoquinone with the nucleophiles were quantified by UV-vis spectrometry, monitoring for the loss of the quinone chromophore. The observed reaction rates spanned three orders of magnitude, from the unreactive amino acids methionine and phenylalanine ($K_{Nu}$ = 0.0002 $s^{-1}$) to the most reactive nucleophile, hydrogen sulfide ($K_{H2S}$ = 0.4188 $s^{-1}$). Analysis of the kinetic data revealed the existence of three categories. The first group, the amino acids methionine and phenylalanine, had rates of essentially zero, while the next group, phloroglucinol and the tertiary varietal thiol 4-methyl-4-sulfanylpentan-2-one, had low reaction rates. The next, more reactive group contained the other varietal thiols and 3-sulfanyl-hexanol and 2-furanmethanethiol. The most reactive substances were the wine antioxidant compounds, $SO_2$, glutathione, ascorbic acid, and hydrogen sulfide. Characterization of the reaction products between the nucleophiles and *ortho*-quinone was performed using HPLC with high resolution MS analysis. The results indicate that the antioxidant compounds, $H_2S$, and wine flavonoids could react preferentially with oxidation-induced quinones under specific conditions, and so may prevent loss of varietal thiol aromas. Future studies will test these comparative rates in wines to evaluate their application to practical winemaking.

*Funding support:* American Vineyard Foundation

## Impact of Different Winemaking Operations on the Concentration of Dissolved Oxygen in Wines

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The importance of oxygen in winemaking is well recognized; however, more information is required regarding the amounts of oxygen dissolved

**Enology — Wine Stability and Oxidation Session – CONTINUED**

at different stages of winemaking and ways to manage that oxygen. This research explored the contribution of different winemaking operations on the concentration of dissolved oxygen in wines. Winemaking operations such as pumping, filtration, continuous tartaric stabilization, and transport of bulk wine were studied. Dissolved oxygen was measured using a luminescence-based meter with temperature compensation (3 LCD-trace Fibox v7; PreSens, Regensburg, Germany). For in-line measurements, a pair of sight glass fittings equipped with low- and high-sensitivity oxygen sensors were placed before and after each piece of equipment to be evaluated. The sensors, in contact with the wine, contain an immobilized luminophore that excites upon exposure to a light beam based on concentration of oxygen dissolved. The operations tested indicate that centrifugal pumps introduce low amounts of oxygen (10 to 30 ppb), while filtration and continuous tartrate stabilization produce much larger oxygen enrichments of 140 to 170 ppb and 2.5 to 2.7 ppm, respectively. When bulk wine was transported in tanker trailers (approximately 3 hr), we observed dissolved oxygen increments that were between 140 and 210 ppb. The effects of using inert gasses on the concentration of dissolved oxygen will also be presented. We expect that these results could help winemakers understand and better handle the contribution of oxygen that different winemaking operations produce.

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## A Mathematical Model of the Oxidation Reactions in Wine

**Roger B. Boulton***
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A mathematical model for the oxidation reactions in wine, based on the iron-initiated pathways presented by Danilewicz (2011), was developed and solved for various conditions. The differential equations for the 18 main reactions were integrated numerically with respect to time for the oxygen uptake examples in that work and the decline in free sulfur dioxide examples in an earlier paper (Danilewicz 2007). The formation of hydrogen peroxide and the subsequent Fenton reactions in the presence and absence of sulfur dioxide, together with the formation of sulfate, were predicted based on catechin as the model substrate. The model is used to estimate the yield of acetaldehyde and to predict the effect of pH on this reaction mechanism.

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## Enology — Phenolics Session

### Effect of Fruit Maturity, Ethanol Concentration, and Maceration Length on Wine Phenolics

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Merlot grapes from a Paterson, Washington, vineyard were harvested 33 days apart on 22 Sept 11 and 25 Oct 11 and vinified to evaluate the effect of grape maturity on wine phenolic composition. At each harvest, half of the must was adjusted to emulate the other harvest's soluble solids content to examine the effect of ethanol on phenolic extraction at different fruit maturities. Chaptalization with a sucrose concentrate (100 Brix) was used to adjust a portion of the first harvest to 25.0 Brix from 20.6 Brix so as not to alter the total volume of the ferment significantly. On the second harvest (24.6 Brix), a portion of the must was bled off and water was added to emulate the soluble solids at the first harvest date (20.6 Brix) without affecting the total volume. To evaluate the effect of fruit maturity and ethanol concentration on seed phenolic extraction, each treatment also included an extended maceration treatment (30 days) and a maceration control (7 days). Each winemaking treatment was performed in triplicate using the same yeast strain and each must was adjusted to the same yeast available nitrogen content. Phenolics were measured using HPLC and tandem mass spectroscopy and spectrophotometric techniques, including protein precipitation for tannins and tri-stimulus values for wine color. A 2.85-fold increase was observed in the anthocyanins between the two harvest dates while skin and seed tannins remained constant. The alcohol conversion factor did not vary, with 20.6 Brix wines producing ~12.0% ethanol (v/v) and 24.6 to 25 Brix wines ~14.5% ethanol. In general, first-harvest wines had significantly higher tannin concentrations and significantly lower anthocyanins and lower polymeric pigments than later wines. The color of second-harvest wines was significantly more saturated and less transparent than that of first-harvest wines. Alcohol concentration was a less important factor for tannin extraction than maceration time. The amount of tannin extracted during extended maceration was the same regardless of fruit maturity or ethanol concentration, suggesting that fruit maturity and maceration length are important factors for tannin extraction.

*Funding support:* Washington Wine Advisory Committee, Washington Wine Commission, and WSU Agricultural Research Center for funds and Chateau Ste. Michelle for fruit

## Enology — Phenolics Session – CONTINUED

### Effect of Ethanol and Press Temperature on the Extractability and Partitioning of Tannins and Anthocyanins in Red Wine

**Meredith Bell** and Douglas O. Adams*
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Tannins and anthocyanins are key elements of evaluating red wine quality; they are responsible for aspects of taste, mouthfeel, and color in red wines. Therefore, understanding factors that influence their extractability is of significance. Only a fraction of the tannin present in berries is extracted during fermentation and many tannins are left tightly bound to the insoluble cell wall matrix of grape berry skins. In this study, the effect of ethanol and press temperature on the extraction and partitioning of grape skin tannins during small-scale fermentation of Cabernet Sauvignon was investigated. Free tannins, both extracted and acetone-extractable, were measured using the Harbertson-Adams assay. Nonextractable, or bound, tannins were measured using an acid butanol ferrous sulfate procedure. The fraction of tannins that tightly binds to the insoluble cell wall matrix of grape berry skins increased during fermentation. However, neither high concentrations (19% v/v) of ethanol or press temperature affected the partitioning of free or bound tannin fractions during fermentation. Likewise, neither high concentrations of ethanol nor press temperature affected the extraction of anthocyanins during fermentation. These results suggest that although tannin binding to the insoluble matrix of grape berries is an important factor in the ability to extract tannins during fermentation, neither increased ethanol nor press temperature contributed to the fraction of tannin extracted into finished wine in this study.

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**Enology — Phenolics Session – CONTINUED**

## Improvement in Protein Precipitation Tannin Analysis by Altering Resuspension Buffer Formulation to Neutral pH

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The analysis of wine tannins has become more commonplace due to their important astringent properties for red wine. There are various methods of analysis, including chromatography; however, protein precipitation assays have earned favor for their simplicity and strong correlation with the sensory perception of astringency. The most widely used protein precipitation method for wine in the United States is a variant on the Hagerman and Butler technique, which allows measurement of both tannins and polymeric pigments. The original method called for high concentrations of sodium dodecyl sulfate (SDS) and an alkaline buffer (triethanolamine, TEA, pH 9.4) to resuspend the tannin-protein precipitate and to support the eventual colorimetric reaction with ferric chloride ($FeCl_3$). However, recently several irregularities were found, including bubble formation (presumably from the detergent SDS), a significant background drift, and loss of reproducibility (presumably due to the alkaline pH). Here, we propose a possible solution: lower the pH to neutral and replace SDS with urea, keeping the original TEA. Forty-nine red wines, primarily Syrah and Cabernet Sauvignon, were tested in triplicate using various buffer formulations (urea/TEA at pH 7, 8, or 9.4 and SDS/TEA at pH 9.4). With urea/TEA at pH 7, the background interference was 33% lower and at pH 8, it was 23% lower than the original method. Tannin concentration was significantly altered by the pH of the resuspension buffer in the following order: pH 8 > pH 7 > pH 9.4. Most importantly, the covariance for tannin analysis was significantly lower ($p$ = 0.006) at pH 7 than at pH 9.4; thus, altering the pH of the resuspension buffer can reduce background interference and improve the reproducibility of the analysis. Also, the incubation time for a steady background reading was possibly reduced to <5 min from 10 min using urea/TEA buffer.

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

## Enology — Phenolics Session – CONTINUED

### Evaluating the Spatial Heterogeneity of Phenolic Profiles in a Pilot-Scale Red Wine Fermentation

**Maximillian Reichwage**, Larry A. Lerno, Charles Brenneman, Cynthia C. Yonker, Ravi Ponangi, Leanne Hearne, Douglas O. Adams, Anita Oberholster, and David E. Block*
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The phenolic component of wine is responsible for its color and taste and plays an important role in the quality of the finished product and associated health benefits. Phenolics are mostly extracted from grape skins and seeds during fermentation. However, the fundamental mechanisms and kinetics of extraction of phenolics from grape skins during red wine fermentations are poorly understood, making informed manipulation of the phenolic composition difficult. Previous studies suggested that temperature seems to have a significant effect on phenolic extraction. The formation of a grape skin "cap" in the upper half of the fermentor during a normal red wine fermentation creates a two-phase extraction system and a temperature gradient as great as 12 to 14°C. To further study the spatial heterogeneity of temperature in a Cabernet Sauvignon fermentation and the associated effect on phenolic profile, a grid of 66 temperature sensors that form a cross-section of a 2000-L tank was assembled. Additionally, samples were drawn from 15 different points near these sensors to measure the gradients in phenolic composition associated with physical matrix differences and temperature gradients. Temperature data were taken every 5 min throughout the fermentation. A full set of spatial samples were taken for chemical analysis twice each day before pumping-over and then every hour for six hours after two key pump-over events. Samples were analyzed for monomeric phenolics, tannins, and color using RP-HPLC, NP-HPLC, phloroglucinolysis, and micro-pathlength UV-Vis. These data will demonstrate how the phenolic composition of the fermentation varies spatially at different points during the fermentation, possibly as a function of temperature, and how this information can be used to optimize color and phenolic extraction in red wine fermentations.

*Funding support:* E&J Gallo Winery

## Viticulture — General Session

### Yeast Assimilable Nitrogen Survey and Amino Acid Profiles in Hybrid Winegrapes

**Amanda C. Stewart** and Christian E. Butzke*
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Yeast assimilable nitrogen (YAN) is an important consideration in fermentation management. The two main sources of YAN are ammonium ions and a-amino acids. Diammonium phosphate is an inexpensive and readily available source of the former and a wide range of commercial products aim to enhance the latter. The impact of prefermentation amino acid profile and concentration on aroma and flavor development in wine is an area of current research and products have been designed to alter the amino acid profile to increase production of fruity esters by certain strains of *Saccharomyces cerevisiae*. This research operates largely under the assumption that proline and arginine are the most prevalent amino acids in winegrapes. Our present work challenges this assumption. We have observed substantially different amino acid profiles in winegrapes common to the Eastern United States. Comparison of profiles from hybrid varieties with *Vitis labrusca* parentage against profiles of *Vitis labrusca* varieties suggests that amino acid profile is heritable. We are also investigating this relationship for *Vitis riparia* hybrids and *Vitis rotundifolia* (Muscadine). Amino acid profile and concentration, as well as ammonium ion concentration, can impact the uptake of amino acids by *Saccharomyces cerevisiae*, and amino acid profile should be considered when designing yeast nutrients for hybrid and native winegrape applications. We have also surveyed YAN in winegrapes across several Midwest and Southern states, observing a range of 89 to 938 mg/L across one vintage, over 30 grape varieties, and four states. For some varieties, average YAN far exceeds our previous recommendations for YAN based on initial sugar content (200, 250, or 300 mg/L at 21, 23, or 25 Brix, respectively). Understanding the differences in amino acid profile and total YAN concentration between hybrids and *Vitis vinifera* is essential to developing targeted fermentation management strategies.

*Funding support:* Frederick N. Andrews Doctoral Fellowship, Purdue University

### Mechanical Harvesting and Sulfite Management to Intensify Tropical Fruity Aromas in Sauvignon blanc Wines

**Paul A. Kilmartin**,* Olga Makhotkina, Mandy Herbst-Johnstone, and Gerard Logan
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We have recently demonstrated that Sauvignon blanc wines with more intense tropical fruit and fresh green aromas are produced from

**Viticulture — General Session – CONTINUED**

machine-harvested grapes, providing an exception to the traditional view that higher quality wines can only be produced from hand-picked grapes. These aromas are due largely to varietal thiols such as 3-mercaptohexanol (3MH) and 3-mercaptohexyl acetate (3MHA), formed from odorless precursors in the juice. A further observation that Sauvignon blanc juices in a more oxidized condition, determined by a greater 420 nm absorbance, have a low varietal thiol potential led us to examine the influence of sulfite additions at harvest on Sauvignon blanc aroma. Grapes and juices were obtained directly from a mechanical harvester at three sites in the Marlborough grapegrowing region. At each site, a well-mixed sample was separated into five 10 kg lots, and sulfur dioxide was added to a concentration of 0, 30, 60, 120, or an extreme 300 mg/L. The sealed buckets were then transported to Auckland for pressing of the juice a few hours later. After settling, the juices were transferred to 750 mL bottles for replicated fermentations at 15°C using EC1118 yeast. The onset of fermentation was related to the free $SO_2$ concentration that remained after transportation, but was only delayed by more than a day for the 300 mg/L $SO_2$ additions. A progressive increase in 3MH and 3MHA concentration was obtained as the juice $SO_2$ at harvest increased to 120 mg/L. Potential roles for $SO_2$ in aroma formation include antioxidant effects, disruption of cellular membranes with increased extraction, and impacts on precursor formation.

*Funding support:* University of Auckland

## Resources for Vineyard Site Selection for Cold-Tender Winegrapes in New York

**Alan N. Lakso**,* Olga Shaposhnikova, Art DeGaetano, Nathan Krause, Richard Piccioni, and Timothy Martinson
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For winegrape growing regions on the cold margins of grape production, it is important to understand and map the patterns of damaging coldest temperatures and to document growing season conditions. Vineyard site selection is a high priority in the rapidly expanding New York State wine industry for experienced and novice growers seeking to optimize current and identify new vineyard sites in regions with many unsuitably cold sites. Important environmental factors (climate, soil, topography, etc.) that influence grapevine winter injury and survival, seasonal development, and fruit quality were integrated with a GIS approach. For New York State, an online GIS-based vineyard site evaluation system (http://www.nyvineyardsite.org) was

created to provide basic site information for site decision support. It includes information on soil suitability, climate, and topography. A continuing study is developing site suitability maps for groups of grape varieties and species for the Finger Lakes Region. Additionally, relationships of topography to temperature patterns in vineyards of the Finger Lakes region with increased resolution were modeled to estimate the winter warming "lake effect" using a temperature database collected on the sub-vineyard scale with temperature loggers. The system represents a valuable tool that helps vine growers in New York State choose a vineyard site.

*Funding support:* New York Wine and Grape Foundation, and the US/ Ukraine Faculty Fulbright Program

## Vine Growth, Nutrition, and Fruit Quality of Cabernet Sauvignon Grafted on Ten Rootstocks in the Paso Robles AVA

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A total of 704 Cabernet Sauvignon (CS8) vines were grafted on 10 different rootstocks and compared to an own-rooted control as part of a new vineyard planting on the terraces above the Estrella River in the Paso Robles AVA. The planting was done on two different sites, each underlaid by a different soil type, within the same vineyard block. We hypothesized that rootstock and soil type would independently influence vine growth, nutrient levels, fruit yield, and fruit quality. Soil type 1, an Entisol, was a sandy soil with reduced water holding capacity due to coarse texture and high gravel content. Soil type 2 was an Alfisol with a sandy loam surface texture over a heavier clay loam subsurface, with higher water and nutrient holding capacities. Three years after planting, the vines exhibited marked differences in growth rate and canopy development, as measured in two consecutive seasons. Tissue analyses performed in 2009, 2010, and 2011 showed differences among rootstocks for all nutrients studied. In most cases, the rootstock rank order was similar for vines grown on both soil types, but there were many exceptions to this trend. Juice from the 2011 growing season was analyzed for degrees Brix, pH, total acidity (TA), α-amino nitrogen, ammonia, yeast available nitrogen, malic acid, and potassium. Juice Brix, TA, and malic acid content varied by both rootstock and soil type. Results of these analyses showed clear differences in juice properties between vines grown on the two contrasting soil types. Notably, pairwise analyses revealed that grapes grown on the Entisol had significantly higher degrees Brix, higher TA, lower pH,

and higher malic acid content than their counterparts grown on the Alfisol. Taken together, these results demonstrate that vine growth and fruit characteristics are influenced by both rootstock and soil type.

*Funding support:* American Vineyard Foundation grant #2011(664)

## Developing a Screen for Assaying Drought Avoidance in *Vitis* Rootstocks

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Plants use a variety of physiological mechanisms to resist drought, including osmotic adjustment, aquaporin-mediated changes in hydraulic conductance, and reduced xylem vessel diameter to resist cavitation. However, when soil water content falls below a critical threshold, yield in crop plants will generally decline regardless of drought avoidance mechanisms. Breeding for drought tolerance may best be approached by selecting for traits that assist in drought avoidance. A root system that is predominantly angled downward, with deep roots and few surface roots, can provide continued access to deep soil water reserves as the soil profile begins to dry, compared to a shallow root system with few deep roots. In rice breeding, populations segregating for deep and shallow rooting have been successfully used to develop molecular markers for drought avoidance root architecture. Similar variability in rooting patterns has been described in grape, but has not yet been exploited in marker-assisted selection. The first step is to obtain a well-resolved root system architecture in an assay with sufficient throughput to be used in the analysis of segregating populations. Methods that provide high-quality data often are suitable only for small studies of a limited number of individuals. We tested a variety of assay methods to optimize root architecture characterization. Factors varied were genotype, container size, container geometry, and duration of growth, and included deep rhizotron containers, root growth pouches, mist propagated herbaceous cuttings, and field excavations. Root architecture was also quantified in a study of interactions between drought and high soil salinity. Among the observations is a strong negative impact on data quality in any method where root systems are constrained by a container, although deep and wide rhizotron containers constrained primarily in one lateral dimension provided excellent characterizations.

*Funding support:* E&J Gallo Winery, California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, CDFA Improvement Advisory Board, and California Table Grape Commission

## Enology — General Session

### Consumption of Grape and Wine Extracts Reduces Fatty Liver in Mice Fed a High-Fat Diet by Promoting Fatty Acid Oxidation

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Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive triglyceride accumulation in hepatocytes. NAFLD affects 30 million adults in the United States and there is a casual relationship between NAFLD and the metabolic risk factors obesity, insulin resistance, dyslipidemia, and nonalcoholic steatohepatitis. We have previously reported that consumption of *Vitis rotundifolia* grape or wine sugar-free extracts markedly decreased dyslipidemia, insulin resistance, and inflammation in C57BL/6 mice fed an obesigenic high-fat (HF) diet. To test the hypothesis that grape phytochemicals will normalize HF diet-induced liver lipid accumulation, we fed 6-week-old male mice one of five diets, low-fat (LF, 10% kcal fat), high-fat (HF, 60% kcal fat), and high-fat diet supplemented with 0.2% resveratrol (RSV), 0.4% sugar-free muscadine grape extract (MG), or 0.4% sugar-free muscadine wine extract (MW), for 15 weeks. We then measured hepatic triglyceride (TG) concentrations and expression of mRNAs involved in lipid metabolism. Consumption of MG, MW, and RSV significantly lowered ($p = 0.001$) hepatic TG levels compared to consumption of HF diet alone. There was a minimal impact on lipogenic gene expression in mice fed grape or wine extract. In extract-fed mice, there was significant up-regulation ($p < 0.05$) of peroxisome proliferator activated receptor-alpha target genes including fibroblast growth factor 21, acyl coA oxidase, and carnitine palmitoyltransferase-I mRNA, suggesting that consumption of the extracts promoted hepatic fatty acid oxidation. In addition, other hepatic mRNAs, Cyp3A11 and NAD(P)H dehydrogenase quinone 1, also increased ($p < 0.05$), suggesting grape polyphenols may also exhibit a hepatoprotective role against xenobiotic and oxidative stress. Collectively, our results demonstrate that consumption of grape and wine polyphenol extracts effectively reduced HF diet-induced hepatic steatosis by promoting fatty acid oxidation, reducing the metabolic complications observed in C57BL/6 mice consuming a HF diet.

*Funding support:* Florida Department of Agriculture and Consumer Services and the Florida Agricultural Experiment Station

## Enology — General Session – CONTINUED

### Phenolic Composition of Malbec: A Comparative Study between Argentina and the United States

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Phenolic compounds play a major role in red wine quality, affecting color, and mouthfeel perception, and aging potential. The objective of this research was to compare the phenolic composition of Malbec wines from California and Mendoza, Argentina. Sixteen vineyards in California and 26 blocks in Mendoza were selected based on their uniformity and regional representativeness. Anthocyanins and low molecular weight phenolics (LMWP) were analyzed by HPLC at approximately six to nine months of aging. Mendoza and California Malbec wines were statistically discriminated when compared on their monomeric anthocyanin, LMWP, and combined phenol profiles. California wines showed a higher concentration of total anthocyanins than the Mendoza wines. Regarding monomeric phenols, Malbecs from California showed higher concentrations of malvidin-3-glucoside and catechin and lower concentrations of gallic acid than the wines from Mendoza.

*Funding support:* Bodega Catena Zapata, Wine Spectator Scholarship, Horace O. Lanza Scholarship, David E. Gallo Educational Enhancement Fund, and ASEV Scholarship

### Temperature during International Wine Transport: Data and Effect

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Wine is shipped mostly in unrefrigerated containers. We explored the following questions: What happens to the product along the supply chain from when the product leaves the winery until it is bought by the consumer? To what conditions is the product subjected during transportation? Do those conditions affect the perceived quality of the product? For over four years, we tracked the temperatures of wine shipments from vineyards and recovered

more than 1000 data loggers shipped from five different wine-producing regions—Argentina, Australia, Chile, South Africa, and California—to more than 42 different U.S. states. A significant number of the shipments were exposed to temperatures of at least 30°C during transportation. We correlated tracking information data with temperature to determine where the wine is at greatest risk of exposure to extreme temperatures (from winery to port, at sea, during transshipment, and from destination port to the importer). We also measured the regulating effect over temperature of using thermal blankets, by measuring the temperature of shipments inside and outside the blanket. Finally, to explore whether typical shipping temperatures damage wine, we built a heating/cooling device to recreate the temperature trajectories recorded in actual shipments. This enabled us to directly compare two bottles of the same wine, one exposed to the recreated shipping temperatures and one not. Five different panels of wine experts blind-tasted such pairs of bottles to see whether they could perceive any differences.

*Funding support:* Supply Chain and Logistic Institute

## Composition of Enological Nutrients and Their Effect on Malolactic Fermentation

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In current enology, it is customary to supply musts with nutrients to prevent sluggish and stuck fermentations. Enological nutrients may be legally added in most winemaking countries and may contain yeast and yeast-derived ingredients, ammonium salts, and vitamins. A recent market analysis reveals over 100 different commercial brands claiming varying compositions and enological advantages. However, in contrast to microbiological media used in laboratories, detailed compositional data about these nutrients is rarely available. This renders the evaluation of their suitability to support wine microorganisms difficult. In this study, six enological nutrients were subjected to a comprehensive analysis. The moisture, amino acid and vitamin profile, elemental composition, and concentrations of inorganic ammonium, primary amino nitrogen, and glutathione were measured. Considerable differences were encountered among the nutrients. The elemental contents differed several-fold for some nutrients (Li, K, Ca, P, Mg, Zn, Fe, and Cu)

**Enology — General Session – CONTINUED**

and exceeded one order of magnitude for others (Na, S, and Mn). Significant differences were also found with regard to the vitamin and amino acid profiles and glutathione concentration. Because of their tedious nutritional requirements, wine lactic acid bacteria were chosen as test microorganisms to study the nutritional quality of the products. Two *Oenococcus oeni* and one *Lactobacillus* strain were grown in a hydroalcoholic test solution with added nutrients at two different titers ($1 \times 10^5$ and $1 \times 10^6$ cfu/mL). The extent of growth stimulation by individual nutrients was strain-dependent. The growth-supporting effect of nutrients was more pronounced at low inoculation densities. Hence, the ability of nutrients to stimulate growth seems more relevant for spontaneous MLF. At high inoculation rates, significant malic acid had already been depleted at the onset of growth.

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## Viticulture — Berry Ripening Session

### Correlation of 3-Isobutyl-2-Hydroxypyrazine and 3-Isobutyl-2-Methoxypyrazine in Winegrapes during the Growing Season

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3-Isobutyl-2-hydroxypyrazine (IBHP) is well accepted as the biosynthetic precursor of the herbaceous-smelling 3-isobutyl-2-methoxypyrazine (IBMP) and may also serve as an intermediate during IBMP degradation. However, the behavior of IBHP during the growing season is not well understood. An improved method for IBHP quantification was developed. Following addition of an $^{2}[H_2]$-IBHP standard, IBHP was isolated by mixed mode cation exchange SPE and silylated prior to GC-TOF-MS analysis. A detection limit of 20 ng/L could be achieved for a 100-mL juice sample. The method was then used to quantify IBHP from fruit set to harvest at sites in the Central Valley of California and in the Finger Lakes region of New York State. IBHP was detectable at the earliest sampling time (four weeks preveraison) and increased to a maximum >800 ng/L in Central Valley Merlot and to >250 ng/L in Finger Lakes Cabernet franc at veraison. IBHP decline occurred following veraison, at least two weeks after IBMP degradation usually commences. Comparison of IBHP and IBMP across sites suggests that IBMP accumulation is dependent on IBHP formation.

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### A Genetic Regulatory Mechanism for Synchronization of Ripening among Individual Berries in Pinot noir Clusters

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Ripening in grape berry clusters starts as an asynchronous process, with individual berries exhibiting various ripening states at the onset of ripening (veraison). Then, the berries will offer a uniform ripening state within the cluster as they reach a ripening plateau near harvest. However, to the best of our knowledge, this statement is scientifically unsubstantiated. In order for the veraison-underripe berries to reach similar ripening states of riper berries, there must be a genetic mechanism to enhance their ripening rates. In Pinot noir asynchronous clusters, we identified four berry classes representative of different degrees of ripening observed at midveraison (50% colored berries) and examined the extent of transcriptional variability in three tissues (seed, pulp, and skin) from veraison to harvest using whole genome microarrays. If

**Viticulture — Berry Ripening Session – CONTINUED**

the underripe and riper berry classes showed significant differences in gene expression at the onset of ripening in the three tissues, the same berry classes showed similar transcriptional states at harvest. To explain this result, we built several models of gene expression dynamics using a two-level comparison integrating berry classes (green, pink, and red), tissues (seed, pulp, and skin), and developmental stages (veraison and harvest). Our models showed that the transcriptional progression in underripe berry classes compared to riper berry classes is enhanced at different ripening stages (postveraison and toward maturity). Other phenological observations clearly showed that veraison-underripe berries have enhanced sugar accumulation during the first three weeks after midveraison, which significantly contributes to early ripening synchrony among berries at the metabolic and transcriptional levels. However, we also found that a significant number of genes will not be synchronized among berry classes before harvest. The cultivar specificity of this regulatory mechanism for ripening synchronization among berry classes within clusters is also discussed.

*Funding support:* Oregon State University

## Hyper-Proline Accumulation in Cabernet Sauvignon Berries Suggests Two Distinct Modalities in Nitrogen Metabolism during Ripening

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In grapevines, nitrogen metabolism plays an important role in regulating vine vigor, crop yield, and the development of berry phenolics. Additionally, the level of yeast-assimilable nitrogen in the grape is a primary factor of its fermentability. Understanding how amino acids accumulate in the developing grape berry is therefore crucial to better wine quality. In a survey of 25 winegrape varieties, two phenological groups were observed: those that accumulate high levels of proline as the berry matures and those that do not. The amino acid profile of Cabernet Sauvignon juice, a hyper-proline accumulating variety, was observed through the 2011 growing season. Preveraison, high concentrations of ammonia were observed to decrease concurrently with proline accumulation. In a second experiment, 10 Cabernet Sauvignon clusters were selected and girdled preveraison, cutting the flow of phloem

**Viticulture — Berry Ripening Session – CONTINUED**

to the ripening berries, and their amino acid profiles were determined throughout maturation. Hyper-accumulation of proline was not observed in girdled clusters. These results suggest that phloem transport of amino acids may play an important role in differentiating the two observed phenological groups. Additionally, the concurrent increase in proline concentrations with declining ammonia concentrations in hyper-proline accumulating varieties suggests distinct amino acid accumulation/biosynthesis modalities between the two phenological groups. Elucidating the physical and biochemical differences in amino acid accumulation in these two phenological groups would help provide crucial insight into fruit quality and the fermentability of finished wines.

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## Enology

### Common and Novel Sanitizers Against Wine Spoilage Yeasts

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The majority of sanitizers currently used in wineries have not been scientifically evaluated against common wine spoilage yeasts found on cooperage and other surfaces in wineries. To attain the elimination of spoilage yeasts, common and novel sanitizers were evaluated under in vitro conditions at different concentrations. Seven strains of the genera *Brettanomyces/Dekkera bruxellensis*, *Zygosaccharomyces bailii*, and *Saccharomyces cerevisiae* were challenged with 150 mg/L sulfur dioxide at pH 3.0, 3.2, or 3.4. Six log reductions and cell killing of *Z. bailii* was caused by 150 mg/L sulfur dioxide at pH 3.0 or 3.2, but not at pH 3.4. *Saccharomyces cerevisiae* strains had almost no reduction (at most 1 log at pH 3.0). *Brettanomyces/Dekkera bruxellensis* strains had log reduction from 3.0 to 1.9 at pH 3.0. At pH 3.2, there was a reduction of 2.5 to 1.2, and at pH 3.4 there was a reduction of 2.4 to 1.0. Dimethyldicarbonate at a concentration of 250 g/L reduced all the strains to zero almost immediately after its addition. However, when 150 mg/L was used, one of the *S. cerevisiae* strains was reduced by only 2.99 log units. The other six strains died almost immediately. This is the first time that dimethyldicarbonate has been tested as a sanitizer and not as a wine sterilant. The standard methodology used may be applied to test different sanitizers without suffering the effects of such compounds per se in wine and without interactions that interfere with the efficiency of the sanitizer.

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### A Novel Heat Maceration Treatment for Pinot noir Must and Its Effect on Wine Phenolics

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The extraction and stabilization of phenolic compounds can cause difficulties for Pinot noir winemakers. A likely contributor to this problem is the asynchrony between anthocyanin extraction, which is largely aqueous and initiates early in pomace contact time, and tannin extraction, which is more strongly ethanol-mediated and hence initiated later in the fermentation period. A heat maceration treatment for Pinot noir must was applied in replicated microvinification of 1 kg batches of fruit. Resulting wines were analyzed for seven phenolic parameters using UV-Vis

**Enology – CONTINUED**

spectrophotometry and modified Somers analysis. The heat maceration method achieved significantly higher anthocyanin and tannin extraction than control ferments and was notable for substantial tannin extraction at the outset of pomace contact time. Histological examination of fresh, heat macerated, and control grape skin provided insights into morphological changes associated with the different extraction outcomes. A maceration technique that offered optimal, early anthocyanin and tannin extraction from Pinot noir must could provide Pinot noir winemakers greater control over fermentation and the possibility of avoiding extended skin contact maceration regimes (e.g., cold soak, extended maceration, dry ice maceration), which consume tank space and risk wine oxidation.

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## Timing and Severity of Regulated Deficit Irrigation on Cabernet Sauvignon. Wines: Interactive Effect of Skin Contact Time

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A 30-year-old Cabernet Sauvignon vineyard in Mattawa, WA, was subjected to four alternative regulated deficit irrigation (RDI) treatments: (1) 100% replenishment of evapotranspiration (100% ETc), (2) 70% ETc, (3) 25% ETc until veraison followed by 100% ETc postveraison until harvest, and (4) 25% ETc. The experiment consisted of a randomized complete block with four repetitions/treatment. To study interactions between the different RDI alternatives and the length of maceration, each vineyard replicate was made into wine with two replicates designated as controls (C, 10-day skin contact) and two as extended maceration (EM, 30-day skin contact). Spectrophotometric analyses (protein precipitable tannins, small [SPP] and large polymeric pigments [LPP], and CIE-Lab tri-stimulus colorimetry) and chromatographic analyses (monomeric anthocyanins, flavonols, vitisins, and polymeric pigments) were performed during maceration and aging. The RDI treatment affected monomeric anthocyanin extraction and color, while the maceration technique primarily affected tannins and polymeric pigment formation. A vineyard × winemaking interaction was only significant for anthocyanin, suggesting that the outcome of extended maceration may depend upon the initial anthocyanin profile of the grapes, as dictated by the

## Enology – CONTINUED

RDI treatment. C wines led to the formation of SPP as a function of monomeric anthocyanin extraction, while EM wines preferentially formed LPP as a function of tannin extraction. EM accentuated tannin extraction more in 25% and 25/100% ETc wines than in 70% and 100% ETc wines. Significantly greater extraction of seed tannins was found with EM, as evidenced by the significantly lower concentration of seed tannins recovered in EM pomace. A statistically higher residual pomace skin tannin and anthocyanin concentration in the EM treatments suggests possible binding of tannins and anthocyanin during extensive skin contact.

*Funding support:* Fulbright Commission, Walter Clore Scholarship, Wine Advisory Committee, Washington Wine Commission, WSU Agricultural Research Center, and Ste. Michelle Wine

### Occurrence of Diglucosylated Anthocyanins as Trace Constituents of the Anthocyanin Profile of cv. Cabernet Sauvignon Grapes and Wines

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Selective ion monitoring (SIM) and precursor ion scan (PI scan) MS/MS techniques were used to examine grape extracts obtained from a 30-year-old *Vitis vinifera* L. cv. Cabernet Sauvignon vineyard located near Mattawa, WA. Sampling points included veraison (26 Aug 2011, ~14 Brix, 2083 GGD), midharvest (12 Sept 2011, ~22 Brix, 2523 GDD), and harvest (12 Oct 2011, ~25.5 Brix, 2901 GDD). A wine made from these grapes was also analyzed at pressing after 30 days skin contact. Twenty-six monomeric anthocyanins were detected in the grapes at veraison. Among them, the diglucoside derivatives of delphinidin, cyanidin, petunidin, peonidin, and malvidin and the acylated derivatives of malvidin and delphinidin are reported here for the first time in *Vitis vinifera* grapes. Some of the diglucosides coeluted with other monomeric anthocyanins; however, a series of PI scans at different collision energies allowed their identification. The anthocyanin profile of the harvest sample was different: the diglucosides of cyanidin, petunidin, and peonidin were absent. As previously reported in Cabernet Sauvignon, pelargonidin-3-*O*-glucoside was detected in the grapes during ripening, although it was absent in the finished wine. Interestingly, trace amounts of the delphinidin and malvidin diglucosides and acetylated malvidin remained in the wine at pressing. To further confirm the presence of these compounds, relevant precursor ion and fragmentation patterns, together with relative elution order, were compared with those observed in Concord (*Vitis labrusca* L.)

**Enology – CONTINUED**

skins, allowing unequivocal confirmation of the presence of the diglucosylated anthocyanins in Cabernet Sauvignon. Although anthocyanin diglucosides are present in trace amounts, their biological significance and potential legal implications as natural components of the anthocyanin profile of Cabernet Sauvignon grapes remain to be investigated.

*Funding support:* Fulbright Commission and Walter Clore Scholarship

### Characterization of *Vitis vinifera* cv. Tannat: An Alternative Variety for Increasing Polyphenol Content in Top Red Wines

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Although the ancient *vinifera* grape variety Tannat is virtually unknown abroad, Uruguay's strategy of promoting it as the country's signature grape is beginning to produce red wines that compete successfully in international wine markets. Previous analysis of polymorphisms at different microsatellite loci showed that Tannat is a highly homozygous variety. At 15 microsatellite loci, the level of homozygosity was 53% for Tannat, in contrast to 6% for Pinot noir, 20% for Cabernet franc and Chardonnay, and 33% for Cabernet Sauvignon. Historically, Tannat was the dominant and almost exclusive variety planted in southwestern France. This geographic isolation, together with the fact that inbreeding is not detrimental for Tannat, may have promoted natural self-fertilization events, which could explain the high homozygosity observed. In this study, we summarize the latest research conducted by our group on this variety, both genetic (genome sequencing and berry development transcriptomic) and chemical (characterization of polyphenolic and aromatic components). We aim to understand the biosynthesis of polyphenols and volatiles during berry development. Tannat has higher polyphenol concentrations and higher antioxidant properties than other red grapes. These characteristics inspired Tannat plantations in many New World regions for blending with other red wines to add structure and improve the aging potential of quality red wines. Genomic and transcriptomic analyses performed on this variety will increase our understanding of its unique characteristics.

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## Enology – CONTINUED

### Sugar-Dependent Nitrogen Demands of *Saccharomyces* and Ramifications for Microbial Wine Spoilage

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Winemakers routinely use nitrogen additions to ensure complete fermentation of high Brix musts, but the relationships between sugar concentration and yeast nitrogen requirements are not well studied. Excessive supplementation of must may also increase *Brettanomyces* spoilage due to residual nitrogen remaining after alcoholic fermentation. To study these relationships, factorial fermentations were performed in synthetic grape juice medium with either 150 or 250 mg/L yeast assimilable nitrogen (YAN) and 230, 250, or 270 g/L fermentable sugar. Two strains of spoilage *Saccharomyces cerevisiae*, one with a low nitrogen demand (ECA5) and one with a high demand (Uvaferm 228), were inoculated at $10^5$ cfu/mL. When fermentation ceased, wines were sterile-filtered and inoculated with *Brettanomyces bruxellensis*. Increased YAN concentrations in media did not substantially impact fermentation rate for a given sugar concentration, but residual nitrogen in these wines was greatly increased. Growth of *Brettanomyces* was not affected by residual YAN, but was hindered at higher ethanol concentrations. In the wines with the highest ethanol concentrations, *Brettanomyces* culturability was dramatically reduced regardless of available nitrogen.

*Funding support:* Northwest Center for Small Fruits Research

### Effect of Wine Matrix on the Analysis of Volatile Sulfur Compounds by Solid-Phase Microextraction-PFPD

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Constituents of the wine matrix, including ethanol, affect adsorption of sulfur volatiles onto SPME fibers, which can impact the sensitivity and accuracy of volatile sulfur analysis in wine. Several common wine sulfur volatiles, including hydrogen sulfide ($H_2S$), methanethiol (MeSH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), diethyl disulfide (DEDS), methyl thioacetate (MeSOAc), and ethyl thioacetate (EtSOAc), were analyzed with multiple internal standards using SPME-GC equipped with pulsed-flame photometric detection (PFPD) at various concentrations of ethanol and other volatile and nonvolatile matrix components in synthetic wine samples. All compounds exhibited a stark decrease in detectability with the addition of ethanol, especially between 0.0 and 0.5% v/v, but the ratio

**Enology – CONTINUED**

of standard to internal standard was more stable at alcohol concentrations >1%. Addition of volatile matrix components yielded a similar decrease, but the standard to internal standard ratio was consistent, suggesting the volatile matrix did not affect the quantification of volatile sulfur compounds in wine. Nonvolatile compounds appear to have negligible effect on sensitivity. Based on analyte:internal standard ratios, $H_2S$, MeSH, DMS, and DMDS can be accurately measured against ethyl methyl sulfide (EMS), the thioacetates with diethyl sulfide (DES), and DEDS and DMTS with diisopropyl disulfide (DIDS) in wine with proper dilution.

*Funding support:* E&J Gallo

## Yeast Community Assembly: Use of GC-MS and PCA to Describe the Developmental Basin Dynamics of Fermentation

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Site-specific yeast interactions influence all wines made with indigenous yeasts. Our studies explore the nature of population growth and the assembly of multispecies ensembles, revealing the extraordinary complexity that arises during biological development. Our current experiments illustrate an ability to tune chaotic developments of growth within the molecular machinery of individuals and adjust species evolutionary rates. At a higher level of organization, that of multiple interacting species, we have found that by varying species introduction sequence and timing, we can create alternative community states from a single set of species. Interestingly, some of these community states can only be produced through a particular sequence of species invasion: they cannot be created from the system parts alone. In concert, these developmental trajectories produce a map outlining plausible developmental routes to alternative end states. We illustrate this using GC-MS and HPLC during the initial phases of fermentation, analyzed using current chemometric techniques to illustrate component trajectories. The formation of specific chemical compounds and system energetics provide us with valuable insight into the timing of introductory sequences and the observation of multiple oscillatory patterns inside the fermentation. Our ability to understand these states and their transitions is vital not only in the winemaking process but also in understanding chaotic developments in the biological world.

*Funding support:* University of Tennessee and Beachaven Winery

## Enology – CONTINUED

### Quantitative and Qualitative Differences in Two Clones of Pinot noir with Different Cap Management Regimes

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This study compared two different cap management regimes on two different clones of Pinot noir grown in the Santa Maria Valley of Santa Barbara County: punch-downs and pump-overs. Each clone/cap management regime was done in triplicate. Total phenols, hue and intensity of color, and nonsoluble solids analysis were performed twice a day for each tank. Adam's tannin assay was performed at three different times: during cold soak, after primary fermentation, and after malolactic fermentation. A tasting panel was also set up, with varying degrees of wine-tasting experience, comprised of students from the Wine Sensory Evaluation class at Cal Poly, San Luis Obispo (spring 2007), local professional winemakers, and tasting-room associates. Of the 34 people on the tasting panel, approximately 56% preferred the clone 4 pump-over wine and approximately 59% preferred the clone 23 punch-down wine. Because the tasting panel preferences were different for each clone, both by a small margin, it would be appropriate to examine the analytical results. Adam's tannin assay analysis is included to show trends in phenolic extraction and anthocyanin binding only. These results were not statistically analyzed. For all analysis performed twice daily, there was either no significant difference between cap management regimes or when there was a significant difference, the most beneficial regime was pump-over. Therefore, the preferred cap management regime for both clones of Pinot noir was pump-over.

*Funding support:* Cambria Estate Winery

### Advantages of Modern Process Control and Fed-Batch Fermentations in Enology

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In recent years, several analytical devices have been introduced that allow winemakers to monitor the progress of alcoholic fermentations. Combined with cooling systems, these technologies can be used to automatically control fermentation rates. Most currently available systems are based on $CO_2$ mass flow, must density, or osmotic pressure measurements. Theoretically, these systems allow determination of sugar concentrations based on initial Brix values and fermentation progress. However, differences in yeast growth,

**Enology – CONTINUED**

transformation efficiency, and by-product formation render this impracticable. We present a system based on FT-NIR spectroscopy combined with chemometric methods that provides accurate real-time information about key parameters throughout fermentation, including glucose, fructose, and ethanol concentrations. In addition to allowing precise control of fermentation rates, real-time knowledge of sugar concentrations permits winemakers to stop traditional batch fermentations automatically at a given residual sugar. More significantly, integrated process control with in-line sugar analysis enables innovative fermentation strategies that include carrying out fed-batch fermentations in enology. For example, a small partial batch of must may be fermented initially until a certain target sugar concentration is reached. The sugar concentration can be kept constant by pumping fresh must into the fermentation vessel throughout the fermentation. This approach is particularly interesting for the fermentation of high-gravity musts (hot climate, late harvest, and icewines), which may lead to hyperosmotic stress in yeast, causing high acetic acid concentrations and potential fermentation problems. To demonstrate the opportunities of this application, the traditional batch fermentation of a must initially containing 340 g/L sugars was compared with a fed-batch approach, where a pump delivered the same must at suitable rates to maintain sugar at 50 g/L. The fed-batch approach showed constant fermentation rates and a reduction of final acetic acid concentration from 1 to <0.2 g/L. Calibrated for malic acid, this system may also find application in conducting malolactic fermentations.

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## Winemaking Yeast Selection for Cool-Climate Chardonnay Wines

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Producing Burgundy-type cool-climate Chardonnay wines is a very specific undertaking. The sought-after complexity in the finished wine is developed and refined during fermentation and aging, including malolactic fermentation. The Institut Français de la Vigne et du Vin (IFV) has developed an original bacterial biomass for Chardonnay winemaking. To complete this research, it was necessary to find a yeast in harmony with the qualitative objectives desired for the final product. Three isolation campaigns were carried out on 2007, 2008, and 2009 vintages to expand the IFV collection of yeasts isolated from Chardonnay. The wineries sampled are well known and never use selected yeasts. Samples were taken from both grapes and must at

## Enology – CONTINUED

different stages of alcoholic fermentation, building an original collection of about 500 strains isolated from Burgundy Chardonnay. Selection was made according to the desired quality for the wine. The main criteria included positive interaction with the bacterial biomass previously selected (49A1), good capacity for producing alcohol, slow fermentation kinetics, average production of acetic acid, and a fruity aroma expression (notably pear, peach, and apricot). Other criteria were also examined, including a high rate of malic acid consumption, low sulfur dioxide production, quantity of biomass produced, capacity for autolysis, and production of glycerol and ethanol. Three strains were selected following these criteria. After selection, genetic characterization showed novel genetic profiles. Two of these strains were produced as active dry yeasts. Trials were carried out in experimental tanks and at three wineries in Burgundy during the 2011 vintage. Similar steps were taken to select a pair of non-*Saccharomyces*/*Saccharomyces* yeasts specifically for cool-climate Pinot noir wines integrating prefermentation cold maceration.

*Funding support:* Department of Agriculture and Lallemand Company

### Quantification, Capture, and Potential Utilization of Fermentation Vapors

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New methods were developed to quantify the loss of alcohol from must during active fermentation. Alcohol and other organic compounds volatilize and are carried away in carbon dioxide produced during yeast metabolism of glucose and fructose. Results are presented for the fermentation of Syrah at constant temperatures (17.5, 22, or 27°C) in 1.8925-L fermentation containers. Results from these experiments are compared to older published data and with emission factors currently used by air-quality agencies to estimate release of organic gas during wine fermentation. Potential uses for the collected volatiles are presented and possible revenue models are discussed for the capture of ethanol and other volatile components.

*Funding support:* Agricultural Research Initiative and California State University

**Enology – CONTINUED**

## Effects of Prefermentation Maceration on the Phenolic Characteristics and Sensory Attributes of Pinot noir

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Pinot noir grapes typically contain low concentrations of anthocyanins in the skins, but high concentrations of seed tannins such as catechin and epicatechin. This unfavorable anthocyanin to tannin ratio can cause significant challenges for producers. Maceration plays a key role in guiding wine quality, with the goal of avoiding wines lacking fruitiness and color or overextracted, tannic wines. Prefermentation maceration was examined as a strategy for improving the quality of Pinot noir wines. Five days of cold soaking at 5°C was included in fermentations of 10 and 15 days, keeping the total duration of maceration, fermentation temperature, and punch-down frequency equal. Spectrophotometry (color intensity and tone), HPLC-DAD (total anthocyanins and catechin), Glories (phenolic maturity), and Folin-Ciocalteu, MCP, and Harbertson-Adams assays (characterization of phenolic composition) were used to analyze the differences between the variations. Additionally, a descriptive sensory analysis was conducted with a trained panel of tasters. Significant increases in extractable anthocyanins were demonstrated post-cold soak, corresponding with comparable increases in color intensity at the end of fermentation. Alhough little change in total phenolic concentration was measured through the addition of a prefermentation maceration, significant differences were observed in the phenolic composition and sensory perception of the resulting wines. Tasters noted an increase in fruitiness and reduced intensity of in-mouth modalities such as tannin perception, bitterness, and astringency.

*Funding support:* Competence Center for Wine Research, State Education and Research Center for Viticulture & Horticulture, DLR-Rheinpfalz

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



## Enology – CONTINUED

### Effect of Storage Temperature and Packaging Type on the Sensory Properties of Cabernet Sauvignon

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The shelf life of wine after bottling or filling is a crucial step toward the final quality seen by the consumer. Yet, in most cases, shelf life is outside the control of the winemaker. Factors like storage temperature and packaging type seem to be important parameters determining the shelf life of wine. We stored Californian Cabernet Sauvignon (vintage 2009) at three different constant temperatures (10, 20, and 40°C) for three months in five different packaging configurations: 0.75-L glass bottles with ROTE screwcaps, natural corks, or synthetic corks and 3-L bag-in-boxes with and without modified atmosphere packaging during filling. During storage, changes in headspace and dissolved oxygen were monitored using noninvasive, luminescence-based sensors. After storage, all samples were analyzed using a generic descriptive analysis for aroma, flavor, taste, and color by 11 trained panelists. Additionally, changes in polyphenolics, volatile pattern, and color were monitored using chemical analyses. Similar to an also-studied Chardonnay but to a lesser degree, significant differences among treatments were found. It also seemed that packaging was less influential in creating differences among samples than temperature.

*Funding support:* Jerry Lohr and Constellation Wines U.S.

### Characterizing the Chemical and Sensory Profiles of U.S. Cabernet Sauvignon Wines

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Cabernet Sauvignon is one of the most reputable red grape varieties grown in the United States. The wines exhibit a wide range of sensory profiles depending on the region, climate, wine style, alcohol concentration, and price. Twenty-four predominantly Cabernet Sauvignon commercial wines from seven AVAs in California and Washington State were selected to encompass a broad range of wine styles; they ranged from U.S. $3 to $125, from 3 to 12 years old, and from 12 to 16% v/v ethanol. The purpose of the study was to characterize the chemical and sensory profiles of U.S. Cabernet Sauvignon wines. A descriptive sensory analysis was performed on all wines in triplicate, using 11 trained panelists. The wines were analyzed for standard chemical parameters and for volatile compounds using GC-MS. There

**Enology – CONTINUED**

were significant differences in sensory attributes driven by price, age, and to some extent, region of origin. Lower-priced Cabernet Sauvignon wines (less than U.S. $30) were characterized by berry, honey, and vinegar aromas and sweet and smooth mouthfeel, with higher concentrations of residual sugar. These wines were generally younger and lower in alcohol. Young Cabernet Sauvignon wines also had significant differences in earthy aromas. Higher-priced Cabernet Sauvignon wines had higher ratings for pepper, vegetal, and barnyard aromas and higher concentrations of methoxypyrazines or *Brettanomyces*-related compounds. These wines were generally older and had higher ratings for bitterness, alcoholic, astringency, and gritty palate descriptors. Californian Cabernet Sauvignon wines were generally high in fresh fruit and barnyard aromas, while Washington State wines had higher ratings for complexity, wood, and alcohol aromas. The results of the study demonstrate the differences in Cabernet Sauvignon wines based on price, age, and region of origin. This work will help the U.S. wine industry define current Cabernet Sauvignon wine styles and identify areas that require further market development.

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## A Genetic Locus Linked to Ethanol Tolerance in *Oenococcus oeni*

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*Oenococcus oeni* is a lactic acid bacterium (LAB) species often employed to carry out malolactic fermentation in wine. *O. oeni* is differentiated from other LAB by unusual acid and ethanol tolerances that contribute to its predominance in wine fermentations. Previous comparative genomic analysis of LAB revealed that *O. oeni* possesses a unique NAD/NADP transhydrogenase system absent in nearly all LAB genomes. NAD/NADP transhydrogenases reduce NADP+ to NADH through hydride transfer driven by an electrochemical proton gradient. These proton-pumping transhydrogenases are a primary source of cytosolic NADPH and have been linked in mammalian systems to protection from oxidative stress. The *O. oeni* transhydrogenase system is comprised of two genes, *pntAB*, encoding the alpha and beta subunits in an operon. Cloning and expression of *pntAB* in *Lactococcus lactis* dramatically improved ethanol tolerance over control strains. The presence of *pntAB* also

## Enology – CONTINUED

correlated with increased resistance to the antimicrobial nisin. These results, combined with the unique presence of these genes in oenococci, suggest a role for the NAD/NADP transhydrogenase system in the ability of *O. oeni* to withstand the highly alcoholic and acidic environment of wine.

*Funding support:* China Scholarship Council

### Kinetics of Ellagitannin Levels in Wine Aged in NIRS Classified Barrels for 24 Months and Sensory Property Impacts

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Eight main ellagitannins (castalagin, vescalagin, roburin A–E, and grandinin) have been isolated from *Quercus* species wood. Their concentrations are highly variable between trees and among different parts of the same tree. Moreover, concentrations in oak barrels also depend on the manufacturing process. These hydrolyzable tannins are extracted during wine aging in oak barrels, but their impacts on the sensory perception of red wine are poorly known. A NIRS apparatus (Oakscan) was used to classify oak staves into four groups according to their ellagitannin concentrations to produce barrels with different polyphenolic indices. The ellagitannin concentrations in the wood were also estimated by HPLC-UV-MS to confirm the NIRS classification. During wine aging, the ellagitannin extraction kinetics was monitored by HPLC-UV-MS each month and the sensory properties of the wines were evaluated by a trained panel after six, 12, 18, and 24 months of barrel aging to estimate the impact of the ellagitannin concentrations. The wood ellagitannin concentrations estimated by HPLC-UV-MS correlated with the NIRS-based classification. In French red wine, the concentrations of wood tannins increase during the first months until they reach a maximum. The date and the concentration of this maximum depend on the barrel's polyphenolic index. The wine aging in the barrel with the highest IP had the highest concentration of ellagitannins in wood after four months aging. Some sensory properties of the wine were impacted by ellagitannin concentrations. Wine with the most ellagitannins was described as having a greater woody and smoked/burned aroma and the least fruity aroma. Moreover, bitterness and astringency were significantly higher in the wine aged in the barrels manufactured with the wood with the highest ellagitannin concentrations.

*Funding support:* Radoux SAS

## Enology – CONTINUED

### Evaluation of the Antifungal Properties of Chitosan: A New Method for *Brettanomyces* Control

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The antimicrobial properties of fungal chitosan, crab shell chitosan, and chitosan-lactate against *Brettanomyces bruxellensis* were investigated. Liquid YM media (pH 3.8, 10% alc.) were inoculated with low populations of *Brettanomyces*: <300, $10^2$, or $10^3$ cfu/mL. Two different *Brettanomyces* strains were used: B1b and B5. The chitosan preparations were added at concentrations of 0 to 8 g/hL. Fungal chitosan and chitosan-lactate were suspended in water while crab shell chitosan was dissolved in 1% acetic acid. To eliminate the potential antimicrobial effect of weak organic acids, 1% acetic acid was used as a control for crab shell chitosan treatments. Eight g/hL fungal chitosan was not sufficient to inactivate *Brettanomyces,* regardless of the yeast population. However, the addition of 4 to 8 g/hL crab shell chitosan or chitosan lactate drastically inhibited *Brettanomyces* growth. Scanning electron microscopy images of the chitosan sediments illustrated differences regarding the interactions of the three preparations with the yeast. While results suggest that chitosan aids in flocculation and sedimentation of *Brettanomyces* cells, preparations also possess unidentified antimicrobial properties. The effectiveness of chitosan against *Brettanomyces* under commercial conditions and its impact on wine sensory properties will be further evaluated.

*Funding support:* School of Food Science, Washington State University, Pullman, Lallemand Inc., and the International Fulbright Organization

### Metabolism of Hydroxycinnamic Acids and Esters by *Brettanomyces* in Different Red Wines

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Depending on the cultivars and other factors, differing concentrations of hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acids) and their corresponding tartaric acid esters (caftaric, coutaric, and fertaric acid, respectively) are found in red wines. Hydroxycinnamic acids are metabolized by *Brettanomyces* to form volatile phenols that spoil wines. However, it is has not yet been determined whether *Brettanomyces* can metabolize the corresponding tartaric acid esters. Populations of $10^4$ to $10^5$ cfu/mL of two strains of *Brettanomyces* were added to commercially produced Cabernet Sauvignon, Merlot, Pinot noir, and Syrah wines. Acidic polyphenolics were analyzed by HPLC

## Enology – CONTINUED

with diode array detection after fractionation by $C_{18}$ solid-phase extraction columns. Prior to inoculation, concentrations of caffeic and caftaric acids were similar in Cabernet Sauvignon and Merlot (15 to 20 mg/L caffeic and 3 to 5 mg/L caftaric) and in Pinot noir and Syrah (12 to 13 mg/L caffeic and 7 to 8 mg/L caftaric). For all wines, concentrations of *p*-coumaric acid (4 to 6 mg/L), coutaric acid (1 to 3 mg/L), and ferulic acid (0.5 to 1 mg/L) were comparable. In all four wines, culturable populations of strain B5 declined similarly over nine weeks while concentrations of hydroxycinnamic acids and tartaric acid esters remained unchanged. Conversely, strain I1a reached populations of $10^7$ cfu/mL in all wines and metabolized varying amounts of caffeic, *p*-coumaric, and ferulic acids after four weeks. Concentrations of tartaric acid esters did not change in wines inoculated with I1a, analogous to B5. Based on these results, *B. bruxellensis* may not be able to hydrolyze the tartaric acid esters of caffeic acid or *p*-coumaric acid. Future research will include two additional strains of *Brettanomyces*. Furthermore, the encouragement of *Brettanomyces* growth through hydrolysis of tartaric acid from caftaric and coutaric acids by other wine microorganisms (*Pediococcus* sp.) will be studied.

*Funding support:* Washington Wine Advisory Committee

### Effect of Interactions among Ethanol, Tannin, and Fructose on the Sensory and Chemical Properties of Washington State Merlot

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The relationship among matrix components and sensory properties of red wine was examined. A Washington State Merlot was dealcoholized and then realcoholized to four ethanol concentrations: 3.15, 8, 12, and 16% ethanol (v/v). Within each treatment, wines were maintained at the original tannin (low tannin) and fructose (low fructose) or brought to high tannin and/or high fructose concentrations (n = 16 solutions). The wines were spiked with constant concentrations of three aroma compounds: 3-methyl-1-butanol, 2-phenylethanol, and eugenol. These wines were then evaluated by a trained panel (n = 10) for the intensity of aromas and flavors (caramel, rose, and clove), tastes (bitterness and sourness), and mouthfeel (astringency and heat). Gas chromatography-mass spectrometry was used to quantify aroma compounds. All data were analyzed using analysis of variance ($p < 0.05$) and Fisher's least significant difference. Ethanol significantly reduced the headspace recovery of all three compounds. The interactions among ethanol, tannin, and fructose varied based on the aroma compound and the ethanol concentration. At standard red wine ethanol concentrations (8 to 16%),

volatile recovery was not influenced by tannin or fructose. However, in low ethanol wines (3.15%), high fructose concentration negatively impacted the recovery of 3-methyl-1-butanol, while high tannin reduced the recovery of eugenol. The trained panel determined that increasing ethanol concentrations increased clove flavor and heat and decreased sourness intensity. High fructose concentration increased rose aroma and flavor scores and decreased clove aroma scores. Tannin concentration positively affected clove flavor while perceived drying and bitterness were impacted by ethanol*tannin. This study demonstrated the complexity of relationships within the wine matrix, thus stressing the importance that winemaking techniques such as saigneé, waterback, and dealcoholization may have on wine quality.

*Funding support:* Northwest Center for Small Fruits Research and the Washington State Grape and Wine Research Commission

## Factors Influencing Heavy Metal Stabilization with Potassium Hexacyanoferrate (II), Divergan HM, and Phytic Acid

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Excessive amounts of heavy metals like iron, copper, and zinc can cause haze problems in wine by complexation with proteins or polyphenols. The potential sources of these metals are fungicides, contact with metal surfaces during vinification, and wine treatments. Their removal prior to bottling is an essential step in maintaining wine stability and quality. Fining agents include potassium hexacyanoferrate (II) (PHCF), phytic acid, and Divergan HM (synthetic polymer based on PVPP). However, the use of fining agents to remove heavy metals from wine is not currently internationally regulated. The aim of this study was to identify factors that support or interfere with fining efficiency. Small-scale experiments were carried out in synthetic medium and real wine, varying pH and the amounts and proportions of iron (II), iron (III), copper, zinc, and manganese. Fining agents were applied at different concentrations and the depletion of metals was analyzed by atomic absorption spectroscopy. The results showed a significant influence of pH on the efficiency of Divergan HM, while PHCF removed most of the metals independent of pH. PHCF needed a certain ratio of $Fe^{2+}$ to $Fe^{3+}$ to work properly. Phytic acid showed the poorest fining performance, with low efficiency for most of the metals analyzed. Due to its polymeric nature and inert behavior in wine, Divergan HM seems to be the best way to remove heavy metals from wine. Although it does not work for manganese and zinc, the most problematic substances, iron and copper, are removed reliably without

## Enology – CONTINUED

the potential health risk of forming hydrocyanic acid as with PHCF. Sensory evaluation of the wines after fining showed no negative effect for all agents. However, wines treated with Divergan HM were judged best by the panel. These results clearly support the application of Divergan HM.

*Funding support:* Competence Center for Wine Research

### Sensory Attributes of Malbec Wine from Seven Districts within the Mendoza Region

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Malbec is the most widely planted winegrape varietal in Argentina. According to the Instituto Nacional de Vitivinicultura, the region of Mendoza produces roughly two-thirds of the nation's wine. Sensory characterization of Malbec wines from large viticulture regions in Argentina has been performed, but the characterization of smaller regions within Mendoza has yet to be quantified. In the press, high-altitude vineyards in Mendoza are often associated with the highest quality Malbec fruit. In this study, 26 vineyard-designate Malbec wines from seven districts within three appellations in the Mendoza viticulture region were analyzed using descriptive analysis. The districts were El Peral, La Consulta, Las Compuertas, Lunlunta, Perdriel, Altamira, and Gualtallary and the appellations were Luján de Cuyo, Maipú, and Valle de Uco. Vineyard elevations range from 930 to 1350 meters. Grapes were harvested between 24 and 28 Brix and wines were made without the use of oak or added tannin. Winemaking conditions were kept constant among treatments. Descriptive analysis was performed with 15 judges and 23 attributes. Significant differences were found among the seven districts. Malbec wines from Lunlunta and El Peral were described by earthy aroma, hot and sweet tastes, and viscous mouthfeel, whereas La Consulta wines were characterized by chocolate aroma and astringent mouthfeel. Malbec wines from Las Compuertas, Perdriel, Gualtallary, and Altamira exhibited wood aroma, but were otherwise balanced wines described equally by the significant attributes.

## Enology – CONTINUED

### Isolation of *Pediococcus* from Oregon and Washington State Red Wines and Their Impact on Wine Quality

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*Pediococcus* species have been isolated from wines worldwide and are generally regarded as being wine spoilage organisms. However, little is known concerning the occurrence of these organisms in Washington and Oregon state wines or their impact, if any, on quality. *Pediococcus* were isolated from Oregon and Washington wines. Seven isolates were identified as *P. parvulus*, one as *P. damnosus*, and one as *P. inopinatus.* Isolates were inoculated into a Pinot noir wine produced at the OSU research winery that had not undergone malolactic fermentation (pH 3.75, no $SO_2$ addition). After significant growth of all isolates, 30 mg/L $SO_2$ was added and the wines were sterile filtered and bottled. Samples were analyzed for several spoilage products including biogenic amines. Most isolates partially degraded malic acid but only three (OW-1, OW-2, and OW-7) completely degraded all malic acid. Low concentrations of biogenic amines were measured in the wines (<3 mg/L total), and only the wine inoculated with *P. inopinatus* OW-8 had >5 mg/L total biogenic amines. D-Lactic acid production varied among isolates, with OW-7 producing the highest concentration at 264 mg/L. Diacetyl concentration also varied greatly, ranging from low (<0.5 mg/L) to high (>15 mg/L) (well above the sensory threshold). Color and polymeric pigments varied, and wines inoculated with OW-7 had 30% less polymeric pigment than the control. Polymeric pigment differences may have been due to variability in the isolates ability to degrade acetaldehyde. Some isolates, including OW-7, reduced the acetaldehyde concentration of the wine. Preliminary sensory analysis of the wines by a winemaker panel demonstrated large sensory differences, with some wines described as having a muted aroma compared to the control while others had intense aromas described as buttery, lactic, Wonder Bread, plastic, musty, and rotten. Differences in mouth flavor and mouthfeel among wines were also noted. More robust sensory analysis is currently being conducted to determine specific sensory impacts of *Pediococcus* spoilage and differences among species and strains.

*Funding support:* Northwest Center for Small Fruits Research

## Enology – CONTINUED

### Controlling *Zygosaccharomyces* and *Brettanomyces* Contamination in Wine

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Sulfur dioxide × temperature or Velcorin were evaluated as means to control growth of *Brettanomyces bruxellensis* or *Zygosaccharomyces bailii* in wine. The addition of 0.5 mg/L $SO_2$ or temperatures of 15°C limited culturability of *B. bruxellensis*. In contrast, 1.0 mg/L molecular $SO_2$ did not affect *Z. bailii*. The addition of 200 mg/L Velcorin to wine containing $10^5$ cfu/mL *B. bruxellensis* or *Z. bailii* caused a loss of culturability within one day for *B. bruxellensis* or within several weeks for *Z. bailii*. While combinations of $SO_2$ and temperature may be more effective as treatment for *B. bruxellensis* than *Z. bailii*, Velcorin may be more appropriate for either microbe.

*Funding support:* Northwest Center for Small Fruits Research

### Consumer-Based Optimization and Willingness-to-Pay for Nutraceutical-Rich Juice Blends

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As consumer awareness of nutraceutical products increases, the value consumers place on health benefits and sensory properties plays a critical role in product development. Concord grape, blackberry, and blueberry juices were blended using formulas created by a ternary-based ABCD mixture design. The resulting 10 juice treatments (three single-component juices, three binary blends, and four tertiary blends) were used for consumer optimization/validation and to determine willingness to pay. Consumers (n = 108) evaluated overall liking on a 9-point verbal hedonic scale. Average liking scores were high for 100% Concord juice (7.79), moderate for 100% blueberry juice (5.47), and low for 100% blackberry juice (2.95). Consumer acceptance was driven by soluble solids, total monomeric anthocyanins, purple color, red color, astringency, sweetness, and grape flavor. For the consumer validation study (n = 78), four optimized blends and three blends from the consumer study were compared. The optimized blend 87% Concord + 13% blackberry juice, created using the maximize desirability function within JMP, had the highest mean overall liking and was used in an experimental auction to determine the emphasis consumers place on health and sensory

attributes. Four auction sessions with 11 to 12 consumers/session were held (n = 47) to elicit willingness-to-pay. Participants in two sessions tasted the product first and then received information about the potential health benefits of anthocyanins (vice versa for the other two sessions). Individuals who tasted the product first and then received information about health benefits had higher willingness to pay for the juice blend than those who received the health benefit information first, which suggests a contrast effect from treatment order. Individuals who exercised more than three times per week were willing to pay more than those who did not exercise as often. Combining the mixture design model and the experimental auction was successful in optimizing and determining willingness to pay for nutraceutical-rich juice.

## Sensory Impact of Interactions among Ethanol, Tannin, and Fructose in a Model Red Wine

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The nature of the wine matrix may affect the perceived sensory quality of the wine. This study assessed the impact of ethanol (0, 8, 10, 12, 14, and 16% v/v), tannin (500, 1000, and 1500 mg/L, grape tannin Biotan), and fructose (200 and 2000 mg/L) concentrations on the sensory properties of model red wines. The intensity of aroma, flavor, taste, and mouthfeel properties of model solutions (n = 36) was evaluated by a trained panel (n = 12) using a 15-cm unstructured line scale. Significant ($p < 0.05$) positive correlations were found between an aroma and its flavor counterpart descriptor: fruity (r = 0.76), woody (r = 0.69), caramel (r = 0.75), sulfur (r = 0.64), herbaceous (r = 0.83), earthy (r = 0.81), floral (r = 0.79), and spicy (r = 0.76). When principal component analysis was applied to the ratings, model wines were differentiated based on factor 1 (floral, fruity, and caramel), factor 2 (earthy and herbaceous), and factor 3 (sulfur). Analysis of variance showed a significant impact of ethanol concentration on these factors, while tannin, fructose, and their interactions had no significant effect. Model wines with lower ethanol concentrations (0, 8, and 10%) had higher floral, fruity, and caramel aroma and flavor scores than model wines with 16% ethanol. Higher scores for earthy and herbaceous aroma and flavor were obtained from model wines with 0, 8, 10, and 12% ethanol than from wines containing 14 and 16% ethanol. In contrast, the lowest sulfur aroma and flavor scores were found in model wines with 0 to 8% ethanol, with increasing sulfur notes observed

## Enology – CONTINUED

with increased ethanol concentration. At 16% ethanol, sulfur notes were strongest. These results indicate the crucial role played by ethanol in modulating the aroma and flavor perception of wine.

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### Microbiological Problems and Off-Flavors during Storage and Aging of Wine in Barrels

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Many wines, especially red wines, are often stored in barrels or barriques to generate more complexity. Wood barrels are situated at the interface between intermediate technologies, involved in the production process, and materials in contact with foodstuffs, required to be inert in relation to their contents. If an off-flavor develops during wine storage, the owner often encounters financial loss. The best-known and perhaps most-distributed off flavor is the "horse sweat" or "Brett off-flavor" produced by *Brettanomyces* species. Other yeasts and bacteria can also influence wine quality by producing off-flavors in barriques. The thorough cleaning of barrels is of great importance in avoiding microbiological contaminations and the production of off-flavors. In some instances, the efficiency of the cleaning processes is low and the next batch of wine develops the same off-flavor. Do relevant microorganisms survive the cleaning process and if so, where? This project focused on barrel wines with off-flavors. The wines were characterized by sensory and/or chemical means. Samples from wine, washing water, and barrels were taken to identify the microorganisms present. Yeasts belonging to the genera *Brettanomyces*, *Debaryomyces*, *Pichia*, and *Zygosaccharomyces* were the most frequent organisms found. Several barriques were sawed and the structure of the inner surface was examined. Caves and blisters behind the toasted surface layer were observed and the same species could be isolated from these structures. Regardless of which cleaning method was used, these organisms survive in these protected niches and could reinfect the wines. Thus, the quality of the toasting process is not only important for aging but also for how the wine quality is influenced by unwanted microorganisms.

*Funding support:* Geisenheim Research Center

## Enology – CONTINUED

### Non-*Saccharomyces* Yeast Affect Pinot noir Wine Volatile Composition

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It was hypothesized that non-*Saccharomyces* yeasts could potentially improve red wine aroma profiles because many non-*Saccharomyces* yeasts exhibit β-glucosidase activity in grape juice. In this study, the impact of selected non-*Saccharomyces* yeasts on the formation of volatile compounds in Pinot noir wine was investigated. Non-*Saccharomyces* species were isolated from an Oregon winery, and species with high β-glucosidase activity, including *Metschnikowia pulcherrim*, *Hanseniaspora uvarum*, *Kluveromyces thermotolerans*, *Hansenula anomala*, and an unidentified isolate #10, were used in fermentations. The selected species were inoculated in high-pressure-treated Pinot noir grape must and held at 9°C for seven days. After the completion of cold maceration, the grape must was fermented with *S. cerevisiae* RC212 at 27°C. The volatile profile was studied using SPME-GC-MS. Fermentation of Pinot noir grapes with the selected non-*Saccharomyces* species resulted in different volatile profiles in the wine. Cold maceration with non-*Saccharomyces* species changed the volatile alcohol, short-chain fatty acid, and ester composition. In addition, cold maceration with *Hansenula anomala* and isolate #10 dramatically increased the concentration of β-citronellol in the wine. However, cold maceration with selected non-*Saccharomyces* species did not increase β-damascenone and β-ionone concentrations. The data demonstrated that non-*Saccharomyces* species have enzyme systems that are active in must and that can alter the terpene alcohol profile of red wine.

*Funding support:* Oregon Wine Board

### Wine Closure Oxygen Permeability and Stale Aldehyde Analyzed by In-Solution PFBHA Derivatization and Solid-Phase Microextraction-GC-ECD

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Stale aldehydes in wine are typically associated with wine oxidation during storage. Pinot noir and Chardonnay wines were bottled with closures having various oxygen transmission rates and then aged four years. Stale aldehydes were derivatized in the wine with *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) at 50°C for 40 min. The corresponding oximes were extracted using DVB-PDMS solid-phase microextraction fiber and analyzed

## Enology – CONTINUED

by gas chromatography–electron capture detection. The limit of quantification for most aldehydes was as low as 0.1 µg/L and the linearity holds at least up to 50 µg/L with $R^2$ of 0.991 to 0.999. The results showed that after four years of aging, the concentrations of some aldehydes varied considerably with different wine closures. Wines bottled with low-density polyethylene screwcaps had the highest concentrations of 3-methylbutanal, propanal, benzaldehyde, phenylacetaldehyde, and methional, which was consistent with this closure having the greatest oxygen permeability. Wines bottled with foil-lined screwcaps had the lowest concentrations of these compounds. Wine closure did not affect the concentration of 2-furfural in the wines, but Chardonnay wine had much higher concentrations of 2-furfural than Pinot noir.

*Funding support:* American Vineyard Foundation

### Yeast and Fermentation Management, Yeast Nutrition, and Managing $H_2S$ Production

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An industry extension project was carried out during vintage 2011 in the Queensland Granite Belt region. The focus of this project was to assess commercially available fermentation nutrient supplements used in many small-scale Shiraz ferments to assess the impact of different nutrient supplements on hydrogen sulfide generation. The project also used a commercially available test kit for assessment of low-level $H_2S$ production. There was little difference seen among treatments during and after fermentation. Some ferments produced slightly more $H_2S$ than others during fermentation, but at the end of fermentation, $H_2S$ concentrations in all ferments were negligible, with sensory assessment also showing no obvious differences in sulfides in any wines produced in this trial.

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

## Viticulture

### Mapping Temperature, Light Exposure, and UV Radiation on the Grape Bunch

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A pilot study was carried out in 2011 during which Chardonnay bunches were assessed for berry temperature, visible light exposure, and UV radiation of berries on various faces of the bunch over the course of the day. Berries exposed to direct sunlight heated up more rapidly than shaded berries, while direct illumination also resulted in exposed berries receiving higher doses of UVB radiation than shaded berries. These factors have implications for berry quality in terms of accumulation of secondary metabolites.

*Funding support:* University of Southern Queensland Centre for Systems Biology

### Mapping Temperature, Light, and UV Radiation of the Grape Bunch: Impacts on Fruit Quality

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A trial was carried out in 2012 in which the impact of bunch exposure on berry temperature, illumination, and exposure to ultraviolet radiation was investigated, using infrared imagery and polysulphone dosimetry. Berries exposed to direct sunlight reached higher temperatures, heated up more rapidly, and cooled more slowly than shaded berries. Direct illumination also resulted in exposed berries receiving higher doses of UVB radiation than shaded berries. Exposed berries also showed differences in various quality measures, including accumulation of secondary metabolites, when compared to shaded berries.

*Funding support:* University of Southern Queensland Centre for Systems Biology

### Grapevine Xylem Phenolic Composition: Correlation with Susceptibility to Pierce's Disease

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Pierce's disease (PD) is a challenging disease for *Vitis vinifera*, with few if any treatments available and limited understanding of natural resistance. *Vitis*

**Viticulture – CONTINUED**

*vinifera* cuttings were collected from two different regions that were warm (nonfreezing winter temperatures) and cold (freezing winter temperatures). Sampling occurred once per season over one year from five *Vitis vinifera* cultivars that spanned three categories of PD susceptibility (least, intermediate, and most susceptible). The concentration of individual phenolics, measured by LC–MS, was highly variable across cultivars and fluctuated by season. Within a variety, the phenolic profile varied seasonally and also by climate. To date, we have identified 25 compounds, including hydroxycinnamates, flavan-3-ols, and flavonols, with at least 15 more compounds unknown. Focusing on the flavonols, as these substances are often noted for antimicrobial activities, it was observed that yearly flavonol concentrations (mg/L) ranged from 50 to 120% more in cold-climate vines than in warm-climate vines and that resistant varieties had higher concentrations than susceptible varieties. Phenolics may impart a protective effect against *Xyllela fastidiosa (Xf)* and cold winter temperatures constitute a stress that triggers higher concentrations of phenolics, protecting the vine from PD by inducing "cold curing" (where *V. vinifera* vines appear to be protected by cold winters). This is the first comprehensive survey of phenolic substances in xylem fluid of *Vitis vinifera*. Our future goals include identification of additional phenolics in the xylem fluid, screening phenolic compounds (and matrices) on *Xf* culture plate bioassays, and induction trials.

*Funding support:* CDFA PD/GWSS Competitive Grant Program

## Method for Rapid Detection of Resistance to Carboxylic Acid Amide Fungicides in *Plasmopara viticola* Populations

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The use of chemical fungicides is an effective strategy to protect grapevines against downy mildew. However, *Plasmopara viticola* is a high-risk plant pathogen for the acquisition of chemical fungicide resistance. The increasing occurrence of carboxylic acid amide (CAA) fungicide-resistant *P. viticola* populations is becoming a serious problem in the control of grapevine downy mildew worldwide. DNA-based diagnosis has become a common tool to evaluate fungicide resistance in obligate phytopathogenic fungi. We developed polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and allele-specific primer PCR (ASP-PCR) methods for rapid detection of resistance to the CAA fungicide Mandipropamid in *P. viticola* populations. PCR-RFLP was used to detect a glycine-to-serine substitution at codon 1105 of the cellulose synthase gene *PvCesA3*, which conferred

CAA fungicide resistance in CAA fungicide-resistant *P. viticola*. The ASP-PCR method is more rapid than PCR-RFLP for detection of the fungicide resistance gene in *P. viticola* populations. Both methods were reliable tools for the rapid and precise detection of CAA fungicide resistance in *P. viticola* populations. Only 4 hours were required from sampling of symptoms to phenotyping of fungicide resistance by PCR-RFLP; 2 hours were required to detect the presence of CAA fungicide-resistance genes.

### Measuring Springtime Temperature Inversion Conditions for Estimating the Potential of Wind Machines for Frost Protection

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Due to the increasingly limited water supplies for sprinkler frost protection in California and elsewhere, there is increased interest in the potential for using wind machines for vineyard frost protection. The ability of wind machines to provide economic frost protection depends on the reliability and magnitude of the temperature inversion conditions during frost events; however,very few growers measure this simple yet highly important variable. Field measurements at two sites on the California Central Coast in 2011 demonstrated the relative ease with which comprehensive, high-density temperature inversion data could be collected at relatively low cost. This data was in turn used to map the inversion conditions on a local scale across multiple frost events. The numerous frosts of the 2011 season created an excellent opportunity to estimate wind machine potential under some of the most severe conditions to be expected in this area. The uncommonly severe frost event of 8 Apr 2011 was beyond the predicted protection range of wind machines, but wind machines would have provided useful protection during the remaining frost events. Importantly, inversion conditions can show significant variation over relatively short distances; therefore, high-density local measurements may provide the most accurate information for addressing individual vineyard decisions. A broader regional survey beginning in 2012 is conducting temperature inversion measurements at 60 locations throughout vineyards in Sonoma, San Luis Obispo, and Santa Barbara Counties of California. The goal of this three-year regional project is to determine the overall potential of using wind machines for vineyard frost protection in these broader regions. The increased adoption of wind machines, where

## Viticulture – CONTINUED

temperature conditions permit, can play an important role in the conservation of limited water resources.

*Funding support:* CDFA Specialty Crops Block Grant Program, American Vineyard Foundation, J. Lohr Winery, and Laetitia Vineyards & Winery

### Comparison of Upward-Blowing and Conventional Wind Machines for Vineyard Frost Protection

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Recently published research has shown that upward-blowing wind machines did not provide any useful degree of frost protection in comprehensive measurements at a single California vineyard site; however, it is unknown if the performance of this type of wind machine might be better under different site conditions. To address this question, changes in vine-level air temperatures caused by both conventional and upward-blowing wind machines were tested at multiple vineyards in the Central and North Coast during the 2010 and 2011 spring frost seasons, with an increased number of locations evaluated in 2012. At each location, air temperatures at the vine were measured at various distances from the wind machine within its theoretical or claimed area of influence and were compared to a nearby reference temperature measured at a similar height outside that area of influence. For each location, linear correlations between the reference temperatures and the temperatures measured near the wind machine were made during nights when the wind machines were not operated. These relationships were then used to predict what the temperature in the vineyard would have been in the absence of wind machine operation on those nights when the wind machines were operated. Lastly, these predicted temperatures are then compared to the actual measured values on nights when the wind machines were operated. By calculating the statistical prediction intervals for each linear correlation, the statistical significance of any deviations in the observed temperature from the predicted values can be calculated. The results of this study will help the vineyard industry attain needed frost protection by improving our understanding of how different types of wind machines perform under a wide variety of vineyard conditions.

*Funding support:* University of California

## Viticulture – CONTINUED

### Assessment of Temperature Spatial Variability in Two American Viticultural Areas of California

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Temperature spatial variability was assessed within two vineyard estates in winegrowing regions of California during the 2011 growing season. Temperature sensors were installed throughout these estates at locations that varied in elevation, slope gradient, slope aspect, and soil characteristics. Two contrasting winemaking regions were selected: the semi-mountainous portion of Lake County (Red Hills AVA) and the dissected alluvial terraces of the Paso Robles AVA in the Central Coast. Climatic conditions of Red Hills are influenced by a rugged relief with elevations between 550 and 750 meters. The climate of Paso Robles is driven by onshore flow which easily crosses the coastal range or comes up the Salinas River Valley, creating occasional morning fog. The average temperature for the season in Red Hills varied between 16.2 and 19.5°C. In contrast, the variation in Paso Robles was between 18.9 and 19.7°C. As a result, these viticultural climates are classified as intermediate (15 to 17°C) to hot (19 to 21°C). Bioclimatic indices usually used at a regional scale were adapted for use at the local scale. They showed an important spatial variability of climatic conditions within both sites. Growing degree days ranged from 1394 to 2053 in Red Hills and 1781 to 1932 in Paso Robles. This range of indices, at a larger scale, would correspond to three climatic zones according to the Winkler categorizations. The spatial variability of the Huglin index was less contrasted because of the lower range of maximum temperatures in both vineyards. The 2011 cool night index analysis showed largely contrasting night conditions for ripening in the Red Hills (7.8 to 18.6°C), while the minimum temperatures were relatively homogeneous in Paso Robles (8.2 to 11.4°C). The position of Paso Robles in a coastal valley enhances night coolness, notably contributing to the synthesis of anthocyanins and flavor compounds, the primary determinants of wine quality.

*Funding support:* ANR-JC Terviclim, GICC-Terdaclim, and the Britanny Region

## Viticulture – CONTINUED

### Hybrid and Selfed Seedling Progenies of *Vitis vinifera* Purpurea Grape Segregate for Tendril Distribution

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Grape varieties typically have green leaves and produce lateral meristems (tendrils and clusters) on at least two successive nodes in three. Morphological markers that distinguish rootstocks and scions would be helpful in identification and reduced lateral meristems could contribute to ease of harvest of rootstock cuttings in the nursery and potentially improve harvest index. To develop improved grapevine rootstocks with purple leaves as morphological markers and with reduced lateral meristems, an elite nematode-resistant rootstock selection ([*Vitis berlandieri* x *V. riparia*] x *V. biformis*) was crossed with a purple-leafed grape selection (*Vitis vinifera* Purpurea) and the purple-leafed selection was self-pollinated. Seedlings from controlled pollinations were grown in a greenhouse and trained up a stake. Starting at the first node in alternate phyllotaxy (that is, a node with a lateral meristem) and continuing for 12 nodes, the presence or absence of lateral meristems was observed and the type of lateral meristem was reported. There were 101 cross-pollinated seedlings and 87 self-pollinated seedlings. The expected pattern of lateral meristem distribution was wild type: two successive lateral meristem bearing nodes followed by a lateral meristem-free node. However, 47 of 87 self-pollinated seedlings showed a non-wild-type pattern, including two or more successive lateral meristem-free nodes (flanked by lateral meristem bearing nodes), off-phase position of the first lateral meristem, and other anomalies. One self-pollinated seedling showed seven successive lateral meristem-free nodes in the alternate phyllotaxy. In contrast, in the hybrid population only 15 of 101 seedlings showed non-wild-type patterns; these included a seedling with alternating nodes bearing lateral meristems (tendrils) and lateral meristem-free. Tendril distribution is under genetic control and the production of extra tendril-free nodes is segregating in these populations, although the genetic control is not fully determined.

*Funding support:* USDA-ARS, American Vineyard Foundation, California Table Grape Commission, California Raisin Marketing Board, and California Grape Rootstock Improvement Commission

## Viticulture – CONTINUED

### Disentangling Dormancy and Cold-Hardiness in Winegrape Cultivars Cabernet Sauvignon and Chardonnay

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Cold-hardiness of bud and cane tissue was monitored throughout para-, endo-, and ecodormancy in field-grown vines using differential thermal analysis to generate lethal temperature exotherms (LTE). Deacclimation and reacclimation rates were measured during ecodormancy to determine the depth of dormancy at which deacclimation became irreversible. Seasonal LTE and ambient temperature data were entered into the published dynamic thermal time predictive model of cold-hardiness and seasonal differences between observed and predicted values were used to assess model accuracy for predicting cold-hardiness under climatic conditions of southwestern Idaho. Cabernet Sauvignon and Chardonnay cane samples were collected monthly or biweekly from the USDA-ARS Parma, Idaho, research vineyard beginning in September, prior to harvest. Percent and onset of budbreak of single-node cane sections (nodes 4 to 8) exposed to 25/20°C (day/night) for 60 days were measured to determine stage and depth of dormancy at each sampling date. Deacclimation and reacclimation were assessed at ecodormancy by determining LTE after exposing canes to forcing conditions and forcing followed by chilling conditions (336 hr at 0°C). Results showed no significant difference between cultivars in cold-hardiness during paradormancy and early endodormancy; however, during most of endodormancy, cultivar differences were significant, with Chardonnay most cold hardy and requiring fewer accumulated chilling units. LTEs were strongly correlated with mean temperature of the 24 hours at day of sampling from July 2011 through January 2012 (Cabernet Sauvignon $r^2 = 0.81$ and Chardonnay $r^2 = 0.87$). We are currently measuring rates of deacclimation as well as evaluating the dynamic thermal time predictive model of cold-hardiness.

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### Management of Cover Crop Residues for the Establishment of Productive Vineyards

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Cover cropping can be an effective tool in managing soil quality and weed control; however, it is often more risky in establishing vineyards where

## Viticulture – CONTINUED

competition for soil moisture and nutrients is a concern. A three-year study was conducted in a newly planted *Vitis vinifera* Chardonnay vineyard in the Willamette Valley to determine if cover cropping could be used to enhance growth and conserve soil moisture. A cover crop of cereal rye (*Secale cereale*) and crimson clover (*Trifolium incarnatum*) was planted each fall and an unplanted treatment was kept free of vegetation and used for comparison. Each spring, the cover crop was mowed and the residue was placed in the tractor row, removed from the plot, or applied as a mulch layer to the vine row at one of two densities: one equal to the residue from the adjacent tractor row and the other at a three-fold rate. After mowing and placing residues each spring, the tractor rows were tilled and kept free of vegetation throughout the summer. Vine growth parameters and tissue nutrients were measured each season and yields and fruit composition in year 3. Mulched treatments resulted in greater vine growth across the three years of the study. Increases in plant growth were likely due to increased soil moisture and reduced soil compaction as compared to unmulched treatments. Mulched treatments had greater leaf chlorophyll, shoot length, fruitfulness, and vine-row root density than unmulched treatments. Tissue analysis of macro- and micronutrients did not result in clear differences, except for higher leaf blade nitrogen at bloom in the 3x mulch treatment. There were no differences in vine yield and fruit composition in year 3. Alternative management of winter annual cover crops appears to be a viable management option for enhancing vine growth in establishing vineyards in western Oregon.

*Funding support:* Northwest Center for Small Fruits Research and Oregon Agricultural Research Foundation

### Responses of Greenhouse-Grown *Vitis* Genotypes to Photoperiod Regimes

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The purpose of this study was to characterize the morphological, physiological, and biochemical changes that occur in grape genotypes in response to short day (SD) photoperiod. Experiments were conducted under greenhouse conditions using cold-sensitive *Vitis vinifera* Cabernet franc (CF) and cold-tolerant *V. riparia* x *V. rupestris* Couderc 3309 (3309C) and *V. labruscana* Concord (CD) cultivars. Potted vines were exposed to SD (8 hr) or long day (LD, 16 hr) photoperiod, for four, six, and eight weeks. Shoot growth, periderm formation, dormancy, freezing tolerance (FT, LT50), and soluble sugar accumulation were examined for each photoperiod duration. Shoot growth

slowed under SD photoperiod in all cultivars. There was increased periderm formation and endodormancy induction, which resulted in increased FT. CD was the first cultivar to initiate these changes in response to SD, followed by 3309C, then CF. FT of all grape genotypes increased by 0.7, 2.0, and 2.7°C after four, six, and eight weeks under the SD regime, respectively. CD reached an LT50 of -10.5°C in week eight, while 3309C and CF reached -12.5 and -8.5°C, respectively. However, FT did not increase under the LD regime and LT50 remained the same among all cultivars, ranging from -6.1 to -8.1°C throughout the experiment. Among all sugars, raffinose showed distinctive responses associated with the two photoperiod regimes. Under the LD regime, raffinose concentrations were low and similar among cultivars; whereas under SD regimes, raffinose accumulation was generally higher than in LD grapevines and the cold-hardy cultivars 3309C and CD accumulated higher concentrations than did the cold-sensitive cultivar CF. Basal leaves and buds accumulated the most raffinose. Results suggest that raffinose synthesis induced by SD alone is an early step in cold acclimation and its differential accumulation in the studied cultivars coincides with their differential acquisition of FT.

*Funding support:* Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center, and Lonz Foundation

## Effect of Alleyway Cover Crop Management on Pinot noir Wine Volatile Composition

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Vineyard floor management is an important aspect of growing healthy grapevines. Very little research has reported the impact of cover crop management on wine volatile composition. It is our hypothesis that cover crop management will affect soil nutrient availability and vine growth, resulting in changes in grape quality and final wine volatile composition. This study investigated the volatile composition of Pinot noir wine made from fruit harvested from vines managed with three alleyway vineyard floor management methods, including clean-cultivated (C), alternating grass and cultivation (A), and solid grass cover (S). The aroma-active compounds were analyzed by stir bar sorptive extraction–gas chromatography–mass spectrometry and solid-phase microextraction–GC–MS. Wine made from grapes grown under clean cultivation had higher concentrations of $C_6$ compounds, including *trans*-3-hexenol, *cis*-3-hexenol, and *trans*-2-hexenol,

**Viticulture – CONTINUED**

which are typically associated with less mature grapes. Higher concentrations of ethyl esters (ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate) were observed in clean-cultivated wine. However, branched-chain ethyl esters, including ethyl isobutyrate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate, were more abundant in wine made from grapes grown under solid grass cover. Meanwhile, cover crop treatments increased the amount of terpene alcohols in wine, including linalool, geraniol, and citronellol. Wine made from grapes grown under solid cover had higher concentrations of β-damascenone. Differences in wine volatile composition may be due to differences in vine size, canopy density, and tissue nutrient status of the vines, which differed between solid cover and clean-cultivated vines. Clean-cultivated vines had larger, denser canopies than solid cover.

*Funding support:* Oregon Wine Board

## Effect of Cluster Zone Leaf Removal on Grape Sugar, Acids, Carotenoids, and Volatile Composition

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High canopy density is common in the cool-climate winegrowing region of western Oregon. Excessive canopy density will produce unbalanced musts, resulting in poor wine quality. Basal leaf removal practices are common in many viticulture regions, leading to improved fruit quality. Selective leaf removal in a grapevine canopy may improve fruit sunlight exposure, increase the photosynthetic activity of the remaining leaves, and can positively influence fruit composition. In this study, the impact of three levels of fruit zone leaf removal, including 0% (control), 50%, and 100%, on sugar, acids, carotenoids, and volatile composition of Pinot noir was studied. Fruit zone leaf removal had no impact on grape total soluble solids, citric acid, tartaric acid, or $C_6$ alcohols and aldehydes of both free and bound forms. Fruit zone leaf removal increased the concentration of several terpenes, including glycosidically linked *cis*-linalool oxide, *trans*-linalool oxide, linalool, α-terpineol, and geraniol. The total amount of terpene alcohol glycosides increased linearly with amount of leaf removal. Fruit zone leaf removal increased the concentration of β-damascenone and the total bound $C_{13}$-norisoprenoids. In addition, fruit zone leaf removal increased the concentration of some carotenoids.

*Funding support:* Northwest Center for Small Fruits and Oregon Agricultural Research Foundation

## Viticulture – CONTINUED

### Comparison of Heat Accumulation and Thermal Distribution between a Hot and a Warm Region for Winegrape Production in California

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To better illustrate the climate of winegrape production regions, heat summation and thermal distribution were determined based on daily and hourly temperatures for the 2010 growing season. Daily minimum, maximum, and average as well as hourly temperatures were collected for Oakville (a moderately warm region III) and Fresno (a hot region V) from CIMIS stations 77 and 80, respectively. Heat summation was calculated as degree days (DD) using the Winkler index and degree hours ($D_xH$) as the product of temperature and hours at that temperature, both with a base temperature of 10°C. Thermal distribution was expressed as $D_xH$ for each degree and for the ranges of <10°C, 10 to 20°C, 20 to 25°C, 25 to 30°C, and >30°C. Regions were compared for the periods of shoot formation (budbreak to anthesis), berry formation (anthesis to veraison), and fruit ripening (veraison to harvest). From anthesis to harvest, daily average temperature remained >20°C in Fresno but <20°C in Oakville. However, maximum daily temperatures were mostly >30°C in Fresno but <30°C in Oakville. From budbreak to harvest, cumulative DD and $D_xH$ for Fresno were much greater than for Oakville. Fresno had similar cumulative DD and $D_xH$ during shoot formation, 140% more DD and 70% more DH during berry formation, and 20% more DD but 4% less $D_xH$ during berry ripening than Oakville. During fruit ripening, when temperatures are most critical for fruit quality, Fresno had less $D_xH$ at 10 to 25°C but more DH at >30°C than Oakville. These results suggest that the heat accumulation calculated as DD from daily temperature can be very different from $D_xH$ calculated from hourly temperature for any region of winegrape production.

### Introducing Degree Hours to Better Interpret Heat Accumulation and Thermal Distribution of Regions and Vintages for Winegrape Production

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Temperature is one of the most important factors influencing vine growth, berry development, and fruit quality in winegrapes. Existing indices for heat accumulation are calculated as summation of daily average temperatures above 10°C from 1 Apr to 31 Oct. Despite their wide acceptance, they

**Viticulture – CONTINUED**

possess some significant limitations, including (1) assuming a symmetric diurnal temperature distribution and overestimating heat accumulation, (2) assuming a growing season from 1 Apr to 31 Oct while harvest occurs long before 31 Oct in warmer regions, (3) assigning equal weight to each degree of daily average temperatures while vine growth, berry formation, and fruit ripening differ in their responses to temperatures, and (4) providing no means to determine thermal distribution for a range of temperatures. Hourly temperatures have smaller variation and provide greater resolution than daily temperatures. Here we introduce hours at a degree ($H_{@}D$) and degree hours ($D_xH$), using hourly temperatures, to better estimate heat accumulation and to interpret thermal distribution. $H_{@}D$ is defined as the number of hours at a certain degree of temperature. $D_xH$ is defined as the sum of the product of $H_{@}D$ and the temperature. $D_xH$ provides a more accurate estimate of heat summation without assuming a symmetric daily temperature distribution. Both $H_{@}D$ and $D_xH$ can be calculated for a given period of time or range of temperatures. Since $H_{@}D$ and $D_xH$ use hourly temperature, it is also possible to differentiate between day and night heat accumulation and thermal distribution. $H_{@}D$ and $D_xH$ provide a better classification and characterization of regions and vintages, a better modeling protocol to predict vine phenology, harvest dates, and fruit quality, and a more accurate evaluation of growing seasons at the key periods of vine growth, berry formation, and fruit ripening.

## Initial Performance of Chambourcin Grapevines Grafted on Selected Rootstocks in the Ozark Mountain Region

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Chambourcin is an important red winegrape cultivar commonly grown in the midwestern United States. Due to concerns regarding long-term vine health because of phylloxera, nematodes, and grape root borer, growers are increasingly interested in using rootstocks for hybrid cultivars such as Chambourcin. Relatively few studies have evaluated the effect of rootstock selection on hybrid cultivars. This study was established in 2005 to evaluate the impact of selected rootstocks on viticulture performance of Chambourcin grapevines. Vines were planted in a Captina silt loam soil in a commercial vineyard near Hindsville, Arkansas. The trellis system was a high bilateral cordon and vines were irrigated using a drip system. Rootstocks included in the study were 3309C, 101-14 Mgt, 5BB, SO4, 1103P, 110R, 44-53, Freedom, and own-rooted Chambourcin. Data were collected during the 2009 to 2011 seasons. Choice of rootstock had little effect on yield and

yield components of Chambourcin grapevines. All treatments produced high yields for this cultivar and training system. Cluster weight was lowest when vines were grafted onto 44-53 rootstock. Fruit composition displayed a small response to rootstock selection. Anthocyanin and phenolic contents of fruit were highest for vines grafted onto 44-53 and Freedom. Vegetative growth, as indicated by dormant pruning weight, did not differ significantly among rootstock treatments. Rootstock has a significant effect on petiole nutrient content. Vines grafted on Freedom, SO4, and 3309C had higher nitrogen than vines grafted onto 110R. Potassium content of petioles was highest when vines were grafted on 1103P and Freedom, with vines grafted on 110R displaying the lowest potassium petiole content. Yeast assimilable nitrogen content was adequate for all treatments. The results to date indicate little benefit of grafting over use of own-rooted vines. However, further evaluation is needed to determine long-term results.

*Funding support:* Missouri Wine and Grape Board, University of Missouri Cooperative Extension Service, Missouri Wine Marketing and Research Council, Missouri Grape Growers Association, and ASEV–Eastern Section

## Diagnosis of Grape Problems with the Vineyard Doctor

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The Vineyard Doctor is the first Internet-based interactive grape problem diagnosis tool. It uses a symptom-based diagnostic approach corresponding to the perspective of a grower observing an unknown problem in the vineyard. A series of illustrated menus progressively guide the diagnosis through selection of the problematic part of the vine (e.g., leaves), characterize the problem (e.g., leaf spots), and describe the symptoms (e.g., size, shape, color, and location of spots). Diagnostic results are presented as an illustrated list of potential causes of the problem, ranked by probability (high, medium, or low). Problem names are hyperlinked to a problem profile page that provides additional information to assist the diagnosis. Problem profiles contain photos and narrative descriptions of primary symptoms or signs, secondary or occasional symptoms or signs, patterns of occurrence, occurrence in the United States, associated risk factors, problems with similar symptoms or signs, and additional actions to assist the diagnosis. The application is formatted for both standard computer screens and mobile devices. The Vineyard Doctor database currently holds more than 150 problems associated with grape production in the United States, including diseases, insects, other pests, nutritional deficiencies and toxicities, physiological disorders, weather-related

## Viticulture – CONTINUED

injuries, and herbicide and chemical phytotoxicity. Members of the eXtension Grape Community of Practice and other experts in viticulture, plant pathology, entomology, and related disciplines contribute, review, and edit the dynamic database for technical accuracy. The Vineyard Doctor is a featured resource of the eXtension Grape Community of Practice and can be accessed at http://www.extension.org/grapes or http://vineyarddoctor.tamu.edu.

*Funding support:* American Vineyard Foundation, Viticulture Consortium West, and Wine and Food Foundation of Texas

### Effect of Deficit Irrigation Frequency on the Mycorrhizal Fungal Community Structure Associated with *Vitis vinifera* Roots

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Deficit irrigation is a powerful tool used to maintain crop yields while enhancing berry quality. However, the use of irrigation, including the amount and frequency of water application, will greatly change the soil environment. Currently, we have a poor idea of how this practice affects other aspects of terroir, namely soil microbiota. *Vitis vinifera* forms important associations with many soil-dwelling organisms, such as arbuscular mycorrhizal fungi. These fungi are root symbionts that improve nutrient and water uptake in many plants and are particularly important under stressful conditions. The identity and functioning of these fungi are highly dependent on abiotic soil characteristics such as water, pH, and nutrient status. We examined whether the timing of irrigation affects the arbuscular mycorrhizal fungi associated with *V. vinifera*. Results from a field trial determined the diversity and identity of the mycorrhizal fungi associated with Merlot and Shiraz vines exposed to either a constant or fluctuating soil moisture profile.

*Funding support:* NSERCDiscovery 2 Grant-MH, NSERC CGS M, BC Wine Grape Council, and Agriculture and Agri-Food Canada's Matching Investment Initiative

## Viticulture – CONTINUED

### Analysis of Cell Viability during Ripening in Healthy and SAD-Affected Grape Berries

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Ripening in fleshy fruits can be considered, at the cellular level, a process of senescence. As ripening progresses, cells in the mesocarp undergo programmed cell death in an orderly manner. Fluorescein diacetate (FDA) is a vital dye that allows differentiation of living cells with intact membranes from dead cells in freshly sectioned grape berries. After staining, the cytoplasm of living cells can be visualized with a fluorescence microscope as a green band surrounding a large central vacuole. Dead cells do not fluoresce and show up as dark spots in the microscope image. The area of living versus dead cells can then be quantified using image analysis software. This method was used to investigate the progression of cell death in ripening berries and in SAD-affected berries at different stages of shriveling. Normally developing berries did not begin to show cell death until about 40 days after veraison. After this point, there was a slow progression of cell death, predominantly in the locular area surrounding the seeds. A large percentage of cells (~80% of the berry area) remained viable well past commercial harvest at 26 Brix. SAD-affected fruit began to lose viability at the same time as visible shriveling of the fruit was apparent, and thus loss of cell viability was not predictive of the disorder, as had been hypothesized. However, SAD-affected berries had accelerated rates of cell death compared with normally developing fruit. The more shriveled the berry, the fewer viable cells it contained. Extensively shriveled berries had extremely low viability (~15%).

*Funding support:* AVF and CCGVE

### Efficacy of Vine Mealybug Mating Disruption with Aerosol-Assisted Delivery

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The vine mealybug (VMB, *Planococcus ficus)* is an important pest of winegrapes in the San Joaquin Valley of California. This study used an aerosol puffer-encased formulation of vine mealybug sex pheromone (lavendulyl senecioate) to provide mating disruption in commercial vineyards. The study compared the efficacy of application rates of zero, 494 g (two aerosol canisters per acre), and 741 g (three aerosol canisters per acre) of active ingredient per acre in two locations. The VMB cluster damage was reduced to 1.08%

## Viticulture – CONTINUED

of the clusters sampled from 4.74% damage in the untreated control. The VMB cluster damage was reduced to 3.32% with two aerosol canisters per acre from 5.82% in untreated control plots. Spatial interpolation analysis was used to create zones of influence patterns and spatial placement optimization of the aerosol devices. Zone of influence patterns gathered from treatment blocks indicated that the areas were fully covered by the VMB sex pheromone at three canisters per acre. Electronic aerosol pheromone distribution and variable timing release over the entire growing season have yielded significant progress in vine mealybug control.

*Funding support:* CDFA Specialty Crops Block Grant and Suterra, Inc.

### Organic Acids and pH of Cabernet Sauvignon Fruit Forced to Ripen under Cooler Conditions in a Warm Region

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A large percentage of California's winegrape crop is produced in warm regions where the fruit usually has high pH and low titratable acidity (TA), producing wines with lower stability and less vibrant color. An experiment was conducted to determine whether shifting fruit ripening to a cooler portion of the growing season with crop forcing could increase organic acids and reduce pH. To force vines, growing shoots were hedged to six nodes and leaves, laterals, and clusters were removed six weeks postanthesis on 24 June 2011. Vines grown under conventional practices were used as a control. Fruit was sampled weekly starting four weeks postanthesis, on 15 June for the control and 30 Aug for forced fruit. Forcing shifted veraison from 25 July to 26 Sept and harvest from 1 Sept to 18 Nov. In both control and forced fruit, pH was lowest at veraison and steadily increased, while the concentrations of tartaric acid, malic acid, and TA were highest before veraison and then rapidly decreased during fruit ripening. The decrease in tartaric acid, malic acid, and TA was slower in forced fruit than in the control approaching harvest. Forced fruit had smaller berries, lower pH, and higher TA at veraison and at harvest. At harvest, forced fruit had a similar concentration of tartaric acid, higher concentration of malic acid, and, in turn, a lower tartaric acid to malic acid ratio due to lower degradation of malic acid. The higher concentration of malic acid contributed to the higher

TA and lower pH in forced fruit. The higher concentration of malic acid without an equivalent increase in tartaric acid resulted in a lower tartaric to malic acid ratio.

*Funding support:* California State University, Agricultural Research Institute and Jordan College of Agricultural Sciences and Technology, Harvey Scholarship

## Insights on Photosynthesis Physiology of Merlot Grapevines (*Vitis vinifera*) Affected by Grapevine Leafroll Disease

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Grapevine leafroll disease (GLRD) is the most complex and economically important virus disease affecting grapevines (*Vitis vinifera* L.). The disease is known to affect vine vigor and longevity and cause significant losses in yield and quality of the fruit. One of the intriguing features of GLRD is that it produces contrasting symptoms in white- and red-berried cultivars. White-fruited cultivars affected by GLRD show mild chlorosis or chlorotic mottling of leaves. In contrast, typical symptoms of GLRD in red-berried cultivars consist of green veins and red or reddish-purple coloration of interveinal areas. These symptoms begin to appear during or soon after veraison and become pronounced as the season advances. The fundamental question in GLRD biology is why disease symptoms are apparent during a specific phenological stage of the crop. As a first step in answering this question, we studied the photosynthesis physiology of cv. Merlot infected with Grapevine leafroll-associated virus 3 (GLRaV-3) in a commercial vineyard. The study was carried out under field conditions between July and September 2009, 2010, and 2011 and changes in pigments, chlorophyll fluorescence, net photosynthetic rate, stomatal conductance, expression of a set of genes related to photosynthesis, and concentrations of soluble sugars and starch were compared between GLRaV-3 infected and uninfected leaves at distinct phenological stages corresponding to preveraison and postveraison. The results indicated that impacts of GLRD on the above indicators associated with photosynthesis physiology were more pronounced postveraison, when leaves displayed GLRD symptoms, than preveraison, when leaves did not display obvious disease symptoms, even though the virus could be detected in leaves at both asymptomatic and symptomatic stages. The results provided

## Viticulture – CONTINUED

an improved understanding of compatible virus-host interactions between GLRaV-3 and grapevine and cause-and-effect relationships of GLRD in a red-berried grapevine cultivar.

*Funding support:* Agricultural Research Center of the College of Agricultural, Human, and Natural Resource Sciences, Washington State University, Washington Wine Commission's Wine Advisory Committee, and USDA-NIFA Specialty Crop Research Initiative Award 2009-51181-06027

### Training Effects on Cayuga White Grapevines in Connecticut

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Cayuga White, an interspecific white hybrid cultivar released by Cornell University in 1972, is widely planted in New England in areas susceptible to winter injury that could be damaging to *vinifera* cultivars. Vines were planted in a commercial vineyard in Connecticut to determine appropriate training systems for the region. The vines were trained to two single-canopy systems, high-wire Hudson River umbrella and low-wire vertical shoot-positioned, and two divided-canopy systems, Geneva double curtain and Scott Henry. The trial is a randomized complete block design with four replications per training system. Vine performance and fruit quality data have been collected for three consecutive years (2009 to 2011). The vertically divided Scott Henry had the highest yields in the first bearing year, but yields consistently declined afterward. The horizontally divided Geneva double curtain had the highest yields in the two subsequent years. Hudson River umbrella was relatively low yielding in all three years. There were few significant differences in fruit quality among treatments and no significant correlations between yield and fruit quality in any years. Most New England vineyards are relatively small, so mechanization of most vineyard activities is rare. Geneva double curtain may be a viable option for production in the area despite the increased trellising and labor costs associated with it.

*Funding support:* USDA Specialty Crop Grant administered by the Connecticut Department of Agriculture

## Viticulture – CONTINUED

### Rootstock and Grape Cultivar Response to an Increasing Soil Water Deficit in Northern Mexico

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Water stress is a limiting factor for crop production in northern Mexico. Grapevine productivity is restricted under drought conditions, diminishing vegetative growth and affecting yield and fruit quality. Rootstocks influence the rate of water uptake and hydraulic lift to cope with the aboveground demand for water. The objective of this study was to investigate the response of two rootstocks and two grape cultivars planted in a field trial established in a sandy loam soil under increasing water deficit. Three irrigation treatments were applied: a fully irrigated control, medium stress (30% less water), and high stress (60% less water). The two cultivars were Merlot cl15 and Chardonnay cl18 grafted to rootstocks 110R and 3309C. Treatments were arranged in a 2 x 2 x 3 factorial matrix and set in the field in a randomized block design with four replications. Plant water status was evaluated through relative leaf water content (RLWC). Data from the second year indicated that 110R was less dry than 3309C for both cultivars in the control and medium stress treatments. However, for the driest treatment, there was no significant difference in RLWC among cultivars and rootstocks. Vegetative growth was also influenced by rootstock and cultivars. Growth on Merlot and Chardonnay increased by 15.2% and 12.7%, respectively, when grafted on 3309C. Growth on 110R may take longer to reach a maximum growth rate. Leaf area index was also greater for both cultivars on 3309C. Under moderate drought conditions, 110R may influence plant drought stress tolerance by maintaining a better leaf water status.

*Funding support:* Universidad Autonoma de Chihuahua

### Anatomical Variations Related to Drought Tolerance in Contrasting Grape Rootstocks

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Drought tolerance is a complex aggregation of traits that allow a given plant species to complete the growth cycle with suboptimal water resources. In order to determine a set of features that could be used to select drought-tolerant individuals in breeding populations, we are contrasting the anatomy

## Viticulture – CONTINUED

of two grape rootstocks: drought-tolerant Ramsey and drought-sensitive Riparia Gloire. General components known to influence water transport and storage are examined in this study. Vessel lumen area ratios of shoot and root sections are being measured using the Adobe PS5 computer program. The number of stomata per leaf area ratios and morphological epidermal differences between the two rootstocks are providing us with a contrasting set of water conservation strategies. We are also comparing pit morphology and vessel/vessel versus vessel/parenchyma relations in woody shoots and roots, which may relate to storage capacity and embolism-discouraging traits. To date, stomata per leaf area counts indicate that there is a significant difference between Ramsey and Riparia Gloire ($p$ = 0.001). Although our measurements of vessel lumen area ratio comparisons were not significantly different, the number of vessels per $cm^2$ of xylem area was different between the two rootstocks.

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

### Influence of Mechanical Canopy Manipulation and Differential RDI Stress on the Phenolic Composition of Syrah Wines

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Canopy architecture, yield components, Ravaz index, berry skin, and wine phenolics of Syrah were measured in response to four canopy management and two differential regulated deficit irrigation (RDI) treatments over two seasons. The control consisted of dormant hand pruning vines to 22 two-node spurs (HP) with no further manipulation. The mechanical canopy manipulation treatments consisted of mechanically box pruning vines to a 100 mm bearing surface, then mechanically shoot thinning to a density of five (CLL), or seven (CLM) count shoots per 30 cm canopy or mechanically box pruning with no further manipulation (CLH). Control vines were irrigated to 70% evapotranspiration (ET) from fruit set until harvest (RDIC). Other vines received 50% ET between fruit set and veraison and 70% ET thereafter (RDIE). The combination of CLM and RDIE resulted in a Ravaz index of 9.9 kg yield/kg pruning weight with 22 tons/ha yield. This treatment combination also increased berry skin phenolics. In the resulting wine, the malvidin concentration in the CLM and RDIE treatment combination

**Viticulture – CONTINUED**

was statistically similar to HP and RDIC. The pigmented polymer and polymeric tannin concentration of CLM and RDIE were optimized with the CLM and RDIE treatment. The study identified a mechanical canopy management method that can be used in combination with differential regulated irrigation stress to reduce input costs, enhance berry skin and wine phenolic composition, and maintain a vine balance that provides for sustainable production.

*Funding support:* American Vineyard Foundation, Agriculture Research Initiative, Bronco Wine Company, E&J Gallo, and Oxbo International

## Development of Quantitative Real-time RT-PCR for Diagnosis and Differentiation of Grapevine Viruses in Vines and Vectors

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Grapevine leafroll disease (GLRD) is considered as the most severe across many grapegrowing regions in the world. Six of the approximately 10 grapevine leafroll-associated viruses (GLRaVs) have been documented in Washington vineyards. GLRaV-3 is the most widespread among the GLRaVs, affecting vine vigor and berry and wine quality. The virus is primarily disseminated via cuttings and spread within vineyards by insect vectors such as mealybugs and scale insects. Due to the lack of curative measures and absence of resistance among winegrape cultivars, preventive measures have to be implemented to contain the spread of GLRaV-3. In this context, accurate and sensitive diagnosis of GLRaV-3 in planting materials and vectors has become a vital component of clean plant programs and disease management strategies. Detection of GLRaV-3 in grapevines has traditionally been achieved by serological methods such as enzyme-linked immunosorbent assay (ELISA). However, positive detection of the virus by ELISA is hampered due to low virus concentration and uneven distribution in grapevines and vectors. To circumvent this limitation, we have developed a reverse-transcription quantitative real-time PCR (RT-qPCR) technique, based on SYBR Green, in combination with melting curve analysis for the detection of GLRaV-3 and GLRaV-2 in wine and juice grape cultivars and in mealybugs and scale insects. The reliability of the assay was further confirmed by sequence analysis of virus-specific DNA fragments amplified in RT-qPCR. A

## Viticulture – CONTINUED

similar approach is being pursued for detecting other GLRaVs with similar efficiency and accuracy.

*Funding support:* Agricultural Research Center of the College of Agricultural, Human, and Natural Resource Sciences, Washington State University, Washington Wine Commission's Wine Advisory Committee, USDA-NIFA Specialty Crop Research Initiative Award 2009-51181-06027, and WSDA Nursery Assessment Funds

### Anthocyanin Composition of Pierce's Disease-Resistant Winegrapes

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The identification and subsequent genetic mapping of *PdR1*, a single dominant gene from *Vitis arizonica* linked to Pierce's disease (PD) resistance, has greatly accelerated a classical breeding program for PD-resistant winegrapes. Using marker-assisted selection (MAS), plants with the resistance gene now exist in vineyards in Sonoma and Napa Counties, Texas, Alabama, and at UC Davis. The breeding program has used a modified backcross strategy and MAS over four generations to produce 97% *V. vinifera* populations with *PdR1*. The resistance source in these populations is from 8909-08, a *V. rupestris* x *V. arizonica/candicans* b43-17 hybrid. The presence of diglucoside anthocyanins is common to American grapevine species, including *V. rupestris* and *V. arizonica*, and has been used as a trait to identify and exclude hybrid grapes from winemaking. This study aimed to determine if the level of expression of diglucoside anthocyanins over four backcross generations diminishes and when diglucoside anthocyanins become undetectable. Clusters were harvested from 5 to 15 progeny at each generation for a total of 89 plants. Each plant used in the study was either directly involved in a cross or served as a proxy for a male parent. A representative sample of 100 berries from each individual was weighed before removing the skins for extraction in a heated and acidified ethanol solution. After the extraction, samples were filtered and analyzed using HPLC. It was expected that the concentration of 3,5-diglucoside anthocyanins would diminish, and eventually disappear, with each subsequent backcross generation and increase in the percentage of *V. vinifera*.

*Funding support:* CDFA PD/GWSS Board and Louis P. Martini Endowed Chair for Viticulture

**Viticulture – CONTINUED**

## Fate of Late-Season Elemental Sulfur Sprays during Berry Ripening and Prefermentation Grape Handling

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Elemental sulfur ($S^0$) residue on grapes is one cause of excessive hydrogen sulfide ($H_2S$) production during fermentation. We have recently reported on a rapid, inexpensive method for quantifying $S^0$, in which $S^0$ is converted to $H_2S$ and then quantified using sulfide detection tubes. This approach has been used to measure $S^0$ residue persistence in field trials in the Finger Lakes region of New York. Microthiol was applied at 6.7 and 5.6 kg/ha in 2010 and 2011, respectively, with sprays ceased at 2, 4, 6, and 8 weeks prior to harvest. $S^0$ residues decreased approximately three-fold faster in late August than in late September in the 2010 trial, potentially because of increased sublimation at higher temperatures. $S^0$ residues at harvest in both years followed the order 2 wk > 4 wk > 6 wk > 8 wk > control. In 2010, $S^0$ residues on the 2-wk treatment were 48 mg/kg, approximately double that observed on the 4 wk treatment. $S^0$ residues over 2 mg/kg were observed in both years even for the 6-wk treatment, which may result in increased $H_2S$ production in some cases. The effects of settling time on $S^0$ residues in must were studied in 2011. Immediately following pressing, 2-wk treatment Riesling grape must contained 24 mg/kg $S^0$. $S^0$ decreased to 4 mg/kg following 1 hr cold-settling, to 0.7 mg/kg after 12 hr settling, and to 0.3 mg/kg after 24 hr settling (final turbidity = 20 NTU). $S^0$ residues were significantly higher in must from the 2-wk treatment than from the 6-wk treatment, even after 24 hr settling (0.7 vs. 0.3 mg/kg), but no significant difference in $H_2S$ formation was observed during fermentation after 12 hr settling. In concordance with previous results, musts that are well clarified prior to fermentation are expected to have inconsequential $S^0$ residues.

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## A Study of Vineyard Vegetation as a Component of Terroir

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Ninety-eight vineyards in the Okanagan and Similkameen Valleys in British Columbia were included in a study of vegetation within and surrounding vineyards as a component of terroir. Plant species diversity was evaluated three times in each vineyard during the 2011 growing season. Grapevine leaf

## Viticulture – CONTINUED

samples were collected during the second and third visits to determine populations of pests and beneficial insects. Later in the season, 14 additional sites were selected for study of native plant communities. These were vineyard-suitable sites but undeveloped and representative of local native ecosystems. Hypothetically, inclusion of plants inherent in local natural ecosystems as vineyard residents can help to integrate native and vineyard landscapes and increase vineyard ecosystem stability by balancing it with the natural environment. The natural and vineyard sites were mapped using geographic information system tools. About 70% of vineyards where populations of beneficial insects were recorded had cover crops composed of at least 10% broadleaf plants at early- and midseason. About 60% of these sites were in close proximity to natural areas. Later in the season, this distribution pattern for beneficial insects was confirmed for only one insect species. Populations of a beneficial *Anagrus* spp. parasitoid wasp remained higher at sites with greater broadleaf plant coverage and most such sites were located near natural areas. Plant species composition in some vineyards changed considerably during the season depending on management practices, some apparently preventing formation of stable habitats for beneficial insects. Plant species diversity in vineyards was low, consisting of a maximum of two to three introduced species. In comparison, natural sites had a minimum of five plant species, observed later in the season. We concluded that populations of some beneficial insects increase when broadleaf flowering plants are resident on vineyard floors and when the grapevines are located near natural areas.

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### Berry Composition and Yield of Cabernet Sauvignon and Malbec in Response to Water Deficit Severity

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Water supply is a production tool used in arid climates to elicit desirable water deficit-related changes in berry composition and yield. However, response to water deficit is known to vary by cultivar. The objectives of this research were to determine whether cultivars differed in their stress responses to water deficit and whether response differences were associated with deficit-related changes in yield and berry composition. Drip-irrigated, own-rooted Cabernet Sauvignon and Malbec grapevines were maintained under mild or severe water deficit during berry development by supplying vines with 70 or 23% of their estimated crop evapotranspiration. The irrigation treatments were imposed after fruit set and sustained until harvest in each of five consecutive

growing seasons. Data collected during the last four seasons showed that both grape cultivars produced ripe fruit under mild and severe water deficit; however, in response to severe water deficit, Cabernet Sauvignon exhibited more severe water stress, greater yield reduction, and less increase in berry anthocyanins and total phenolics than did Malbec. Predawn and midday leaf water potential were similarly related in both cultivars, but differences between cultivars in their physiological response to drought were apparent from the unique relationships among soil moisture, midday leaf water potential, and stomatal conductance. The physiological adaptations of these cultivars to severe water deficit revealed a combination of drought avoidance (stomatal regulation) and drought tolerance (reduced vegetative growth). Under the arid conditions of this study, optimum yield and berry composition were achieved in Cabernet Sauvignon at less severe levels of water stress than were optimum for Malbec. Results from this research present new information about cultivar differences in response to drought and their relationship with berry composition and yield and practical information for optimizing irrigation strategies for these cultivars under arid conditions.

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## Weed Management Comparisons in Organic Vineyards: Economics and Efficacy

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Weed management can amount to substantial costs in organic vineyards because of the lack of cost-effective, reliable, and organically acceptable herbicides. Information is needed to help growers select the most economic and environmentally sustainable weed control practices for their vineyards. On-farm studies were conducted in 2010 and 2011 in organic raisin and wine-grape vineyards in the San Joaquin Valley. Weed management treatments included steam, French plow (raisin vineyard only), Bezzerides cultivator, and an organic herbicide (Greenmatch). These treatments were followed by a second treatment of steam, organic herbicide, or hand weeding. Nonweeded controls were also included. Data on weed control, grapevine growth, crop yield, and fruit composition were collected. Time needed to hand-weed each plot was recorded. The mechanical treatments (French plow and Bezzerides cultivator) provided the best weed control and were the most economical. The steam and organic herbicide treatments provided only three to four weeks of weed suppression and cost two to four times more than the

## Viticulture – CONTINUED

mechanical treatments. However, in general, no treatments affected vine growth or crop yield or quality, suggesting that established vineyards may have high weed thresholds. Nevertheless, growers should manage weeds to reduce their potential interference with cultural practices and to prevent them from serving as alternate hosts to other pests and diseases, reducing irrigation efficiency, adding seeds to the soil seedbank, and lowering the aesthetic quality of a vineyard.

*Funding support:* Viticulture Consortium West, California Raisin Marketing Board, CSU–ARI, and Propane Education and Research Council

### A Collaborative Research and Extension Outreach Model: The Grape Community of Practice

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In 2009, the grape community of practice (GCoP) was formed within the eXtension system, which is an Internet-based collaborative environment where land grant university content providers exchange objective, research-based knowledge to solve real challenges in real time. The GCoP leadership team consisted of members of the Extension and Outreach Education Committee of the National Grape and Wine Initiative (NGWI). This committee addressed an overall priority of NGWI to enhance outreach education for grape producers by targeting development of a GCoP for the eXtension system. The GCoP launched www.eXtension.org/grapes in 2010 and a companion site in 2011 called www.eViticulture.org. To date, the GCoP has 77 members from cooperative extension, research, and industry in 31 states and Canada who interact through a variety of online methods to collaboratively create content. All content is reviewed by experts within our community of practice. More than 100 articles have been translated into Spanish to reach a broader audience. Data collected from Google Analytics show that content from the eXtension grape site was viewed more than 135,000 times during 2011. After successful establishment of the GCoP, NGWI strongly encouraged other proposals to become linked to the GCoP for part of their outreach component. Through this process, two funded projects in 2011 included the GCoP as part of their outreach component: "Accelerating grape cultivar improvement via phenotyping centers and next generation markers" (Reisch) and "Northern grapes: Integrating viticulture, winemaking, and marketing of new cold-hardy cultivars supporting new and growing rural wineries" (Martinson). Expected outcomes for these projects and the GCoP

**Viticulture – CONTINUED**

are to create comprehensive online resources for research-based information by providing viticulturists access to content addressing all aspects of grape production, linking to all state-based sites, integrating projects to increase networking and collaboration, and reducing information redundancy across the country.

*Funding support:* USDA-NIFA-SCRI

## Nitrogen Status and Fermentation Dynamics for Merlot on Two Rootstocks

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Seasonal nitrogen (N) application and two rootstock cultivars that differed in potential growth rate (vigor) were compared for vine biomass, vine N status, fruit yield and quality, must amino-N composition, and fermentation dynamics. The rootstock 1103 Paulsen (*Vitis berlandieri* x *rupestris*) has a root system that tends to provide high shoot growth. The other rootstock 101-14 Millardet et de Grasset (*riparia* x *rupestris*) produces a root system associated with lower shoot growth. Vines were given N in the fall (FF) and spring (SF) or no nitrogen (NF) in an established Merlot (clone 1) experimental block in Oakville, California. Merlot grown on the 1103P vines had two times the yeast-assimilable nitrogen (YAN) concentration as Merlot grown on the 101-14 Mgt vines. In essence, the difference in nitrogen metabolite levels between the two rootstocks suggests 1103P roots may be more effective at absorbing and/or translocating N whether it is from fertilizer or the soil mineral pool. The data also suggest that Merlot vines on 1103P rootstocks in the Napa Valley may require little to no N supplementation while Merlot grown on 101-14 Mgt may require N supplementation to avoid slow or stuck fermentations. Furthermore, within rootstock treatments, YAN levels alone did not explain observed rates of fermentation, suggesting that other metabolites may play an important role in fermentation dynamics.

*Funding support:* American Vineyard Foundation, California Competitive Grant Program for Research in Viticulture and Enology, and Maynard A. Amerine Endowment

## Viticulture – CONTINUED

### Small Differences in Temperature Interact with Solar Radiation to Alter Anthocyanin in Grapes

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Anthocyanins are important components of all dark-colored fruit and are crucial for red winegrapes. Despite a century of research, we still lack a concrete, mechanistic understanding of solar radiation and temperature effects on anthocyanin accumulation and composition. Our aim was to elucidate the mechanistic response to microclimate of anthocyanin metabolism in *Vitis vinifera* cv. Merlot, in this case solar radiation. Unlike previous work, this study measured temperature, radiation intensity, and relative humidity continuously for the duration of treatment. Research was conducted in a vineyard near Prosser, Washington. Treatments included clusters that were (1) control-shaded (CS, ambient temperature), (2) control-exposed (CE), (3) light-excluded (LE), and (4) aspirated light-excluded (ALE). Aspiration was initiated and terminated dynamically in reference to the temperature of CS clusters as estimated by averaging berry skin temperature from multiple berries per cluster. For LE and ALE, randomly selected healthy clusters were shaded at the onset of veraison (one to two berries turning color) by polypropylene enclosures comprised of a white exterior and a flat black interior. All experimental clusters were located on the west aspect of the canopy. Treatments were imposed until a composite berry sample from untreated clusters reached commercial ripeness (~23 Brix). Harvested grapes were chemically extracted and extracts were analyzed for individual anthocyanins by HPLC/DAD. All 15 anthocyanins previously reported in Merlot grapes accrued in all clusters, indicating that no individual accumulations were terminated by light exclusion during ripening. Overall, both LE and ALE clusters accumulated less total anthocyanin (86.8 mg malvidin-3-glucoside equivalent/100 g berry) than the controls (160.2 mg malvidin-3-glucoside equivalent/100 g berry). Proportions of individual anthocyanins were altered by light exclusion. The ALE clusters had fewer total anthocyanins (75.4 mg malvidin-3-glucoside/100 g berry), but higher percent acylation (52% total anthocyanins) than the other treatments (CS, 36%; CE, 37%; and LE, 42%). This work contributes toward our knowledge of environmental stresses and anthocyanin development in grape berries.

*Funding support:* USDA-ARS

## Viticulture – CONTINUED

### Effect of Nitrogen Availability at Veraison on Canopy Efficiency and Fruit Quality at Harvest

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The nitrogen status of grapevines affects canopy efficiency, with excessive nitrogen resulting in vegetative and reproductive competition that impacts fruit development and delays fruit ripening. Our objective was to increase canopy photosynthetic efficiency (source) at veraison, increasing foliar nitrogen concentration and evaluating the potential effect on photosynthetate production and translocation to the ripening clusters (sink). At veraison, the canopy was manipulated by leaf area reduction treatments (0, 33, or 66% of the total) and nitrogen was supplied with a spray application of 1% urea. A complete randomized design was arranged using Chardonnay vines trained as VSP at the Southwest Michigan Research and Extension Center, Benton Harbor, MI, during summer 2011. Leaf photosynthesis (Pn) was measured in different position of the canopy (nodes 4–5, nodes 10–15, and apical nodes) up to three weeks after the application of treatments. Diurnal Pn was affected by the nitrogen application in apical leaves when compared to unsprayed control (+15%). In older basal leaves (nodes 4–5), Pn decreased, particularly under the defoliation treatments (33 and 66%). In vines where 33% of the leaf area was removed, we observed Pn compensation only at nodes 10–15, with an additional Pn increase in vines where nitrogen was applied. Instead, increasing defoliation to 66% reduced Pn on basal leaves and N application reduced chlorophyll fluorescence and SPAD index. At harvest, fruit basic chemistry (Brix, pH, TA) was not impacted by the nitrogen application, but yeast assimilable amino acids concentration increased. There is a positive effect of nitrogen supply and leaf area reduction (30%) on photosynthetic efficiency, but only at the apical position (recently developed leaves), which did not compete (sink) or support (source) the developing cluster during ripening.

*Funding support:* Michigan Grape and Wine Industry Council and Project GREEEN at Michigan State

### Effect of Yield Manipulation on the Aroma Composition of Central Otago *Vitis vinifera* L. cv. Pinot noir

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The Central Otago region of New Zealand is renowned for the production

## Viticulture – CONTINUED

of premium Pinot noir wines. Various volatile compounds are responsible for Pinot noir's complex aroma and play a major role in the perception of its quality. Pinot noir grapes are particularly sensitive to yield and in the Central Otago region it is generally considered that restricting yields to 3 to 4 tonnes per hectare (t/ha) results in higher quality wines. While it is known that high yields (>8 t/ha) in this region may result in undesirable aromatic characteristics, little is known on how aromatic composition changes with yields varying from 3 to 7 t/ha. To understand the influence that cropping levels may have on the aromatic composition of Pinot noir wines from this region, nine nominated plots within three vineyards under the same viticultural practices were bunch-thinned to achieve yields from 2 to 7 t/ha across three consecutive years (2009 to 2011). Grapes were hand-harvested at 24 Brix and wines from each block were made on a commercial level by the same winemaker in stainless-steel tanks under the same conditions. Samples for analyses were taken prior to malolactic fermentation and oak exposure. Over 60 volatiles, including terpenes, norisoprenoids, esters, $C_6$ alcohols, higher alcohols, fatty acids, aldehydes, and volatile phenols were analyzed using both liquid:liquid and solid-phase microextraction and later injected onto a GC-MS. Significant differences were observed in concentrations of aromatic compounds in wines made from different yield treatments within each vineyard's data set and within a given year. However, no clear trends could be determined for the investigated aroma compounds between years both within a vineyard site and between the sites.

*Funding support:* New Zealand Ministry of Science and Innovation

### Water Footprint of Chardonnay Grown in the Carneros District

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The water footprint is the sum of water required to produce a product and is usually expressed as $m^3$ of water $tonne^{-1}$ of product for agricultural/horticultural crops. The global, average footprint for grapes has been estimated to range from 608 to 869 $m^3$ $tonne^{-1}$ of fruit. An irrigation study was conducted in a Chardonnay vineyard located in Carneros across eight years. Five irrigation treatments were imposed the first four years of the study: applied water at 0.25, 0.5, 0.75, 1.0, and 1.25 of estimated vineyard evapotranspiration ($ET_c$). Three treatments were imposed the last four years: no applied water and applied water amounts at 0.5 and 1.0 of estimated $ET_c$. Soil water content was monitored with a neutron probe using access tubes located in ¼ of an individual vine's rooting zone (6 tubes $vine^{-1}$) down to a depth of 3 m with six replicates per irrigation treatment. Applied water was measured with

**Viticulture – CONTINUED**

inline (in the drip line) water meters. The water footprint ranged from 130 to 429 $m^3$ water $tonne^{-1}$ during the first four years of the study across treatments. During the 1999 growing season, $ET_c$ values of the 0, 0.5, and 1.0 irrigation treatments from budbreak to 31 Oct were equivalent to 249, 345, and 450 mm of water, respectively. Yields of the 0, 0.5, and 1.0 irrigation treatments were 10.9, 14.1, and 14.8 tonnes $ha^{-1}$, respectively. Based upon the yield and $ET_c$ data in 1999, the Chardonnay water footprint values for the 0, 0.5, and 1.0 treatments were 226, 244, and 301 $m^3$ $tonne^{-1}$, respectively. The above demonstrates that the actual water footprint of irrigated grapevines is dependent upon applied water amounts and final yield. The water footprint values presented herein also illustrate that values given by others for grapes may not be realistic.

*Funding support:* American Vineyard Foundation

## Reexamining the Roles of Xylem Flow and Transpiration in Grape Ripening

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Water determines the concentration of sugars, acids, phenolics, and other compounds in grapes. Imbalances in water content can cause berry cracking or shrinkage. Previous studies often neglected the effects of fruit transpiration and xylem flow on berry water balance after veraison. We hypothesize that both xylem backflow and fruit transpiration, two water-disposing methods of berries, play roles in berry growth and sugar accumulation. Clusters of three cultivars in the vineyard were treated with either xylem removal, transpiration removal, or the removal of both xylem and transpiration. Berries without xylem and/or transpiration accumulated sugar less rapidly than controls. Berries without transpiration gained more than or the same weight as controls and the incidence of berry cracking was higher, especially in Syrah. Berries without xylem gained less weight than controls. In addition, to study the role of xylem backflow, berries were collected from potted vines which had their roots pressurized to stop backflow. Weight gain, sugar accumulation, and deformability (related to shrinkage) were measured. Without xylem backflow, berries expanded more, accumulated less sugar, and were less deformable than controls. Likewise, berries were attached to a customized "berry pressurizer" to test their vulnerability to cracking. Syrah and Concord were more vulnerable to cracking than Merlot; Syrah exhibited a window of vulnerability during ripening but Concord and Merlot did not. We conclude that berry transpiration has a significant effect on the rates of sugar accumulation and berry growth. Without transpiration, berries gain

## Viticulture – CONTINUED

weight more rapidly, but accumulate sugar more slowly, than those with transpiration. The direction of xylem flow is essential to sugar accumulation. Xylem backflow facilitates sugar accumulation and protects berries from cracking. The vulnerability to cracking varies depending on cultivar and growth stage.

*Funding support:* Northwest Center for Small Fruit Research, Rhone Rangers, and Washington State University

### Impact of Viticultural Practices on Fruit Quality of Cabernet franc Grown under Michigan Cool-Climate Conditions

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The acreage of *Vitis vinifera* in Michigan has steadily increased by 10% over the past 15 years and Cabernet franc is the most widely planted red grape variety after Pinot noir. However, fruit maturation of red varieties is challenged by the short, cool growing season and growers often struggle to achieve the desired fruit quality at harvest (basic fruit chemistry, anthocyanins, phenolics, and flavor compounds). Different levels of crop reduction and leaf defoliation were investigated in complete randomized block design experiments during 2010 and 2011 at the Southwest Michigan Research and Extension Center, Benton Harbor, MI, on mature 20-yr-old Cabernet franc vines grafted on 3309C, spur pruned, and trained as VSP. Three crop levels of 12, 24, and 48 clusters per vine were imposed at fruit set, preveraison, veraison, and postveraison and basal defoliation (nodes 1 to 6) was performed at the same time. Canopy growth (shoot length, diameter, and leaf area) was measured weekly until veraison. In 2010, vine growth (indexed as winter pruning weight) and basic fruit chemistry were not significantly affected by the treatments. However, phenolics and anthocyanins increased when crop reduction and leaf pulling treatments were applied at fruit set. In 2011, vine size was not affected by the treatments, while basal leaf removal increased anthocyanins and phenolics only in low-yielding, but not in high-yielding, vines. Currently, flavor compounds (IBMP and rotundone) are being measured. Yield per vine impacted anthocyanins and phenolics but not basic fruit chemistry measures. Timing of crop reduction had a minor impact on fruit chemistry, while leaf pulling increased fruit quality in low-yielding vines.

*Funding support:* GREEEN Project at Michigan State and Michigan Grape and Wine Industry Council

## Industry

### The Spectro-Electro Array: A Novel Platform for the Measurement of Secondary Metabolites in Botanicals, Supplements, Foods, and Beverages. Part 1: Theory and Concepts

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Plants contain a diverse range of secondary metabolites, many of which are purported to offer health benefits. The challenge for the analytical chemist in the measurement of these compounds is two-fold: first, to develop assays that can accurately discriminate between compounds that often have similar physicochemical characteristics and, second, to analyze such compounds in complicated matrices including botanicals, supplements, foods, and beverages. Gradient HPLC with diode array detection (DAD) is often used to determine natural products. However, this approach often suffers from a lack of specificity, as compounds with similar structures may coelute chromatographically and cannot be deconvoluted spectrally. Furthermore, this technique lacks sensitivity, limiting its use for the study of natural product metabolism in animals and humans. Coulometric electrochemical array detection, in contrast, is selective, being able to distinguish between subtle changes in chemical structure, and, with sub-picogram limits of detection, can be used to examine natural product absorption and metabolism. The combination of DAD and coulometric electrochemical array detection extends the range of compounds measured by either technology alone. The concept behind the spectro-electro array will be discussed and the power of this approach will be illustrated using a global polyphenol method capable of measuring ~50 analytes simultaneously.

*Funding support:* Thermo Fisher Scientific

### The Spectro-Electro Array: A Novel Platform for the Measurement of Secondary Metabolites in Botanicals, Supplements, Foods, and Beverages. Part 2: Metabolomics

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Plants contain a diverse range of secondary metabolites including polyphenols, alkaloids, and terpenoids. Although these compounds are not involved in normal growth, development, and reproduction, they still play a crucial role in the organism. For example, polyphenols act as pigments and can protect against disease. Secondary metabolites are also thought to be responsible

**Industry – CONTINUED**

for the purported health benefits associated with the consumption of botanicals, supplements, some foods, and beverages. Interestingly, polyphenols are also associated with the quality and sensory characteristics of wine. Gradient HPLC with spectro-electro array detection is selective, sensitive, and can be used to measure hundreds of known and unknown secondary metabolites in a sample. Such metabolite profiles contain a wealth of useful information. Changes in the pattern of metabolites, when evaluated using chemometric modeling software, can be used to study product adulteration, contamination, composition, and stability. In wine and fruit juice, the method can distinguish the effects of growing region and differences between varietals. The application of this metabolomic approach to wine, fruit juice adulteration, and botanicals will be discussed in greater detail.

*Funding support:* Thermo Fisher Scientific

## Validating the Oxygen Performance of a Wine Closure

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Oxygen plays a key role in wine chemistry and aging as it affects the stability and sensory properties of wine. Among the current choices of wine closures, the discussion of oxygen permeability has come to the forefront. However, there is little agreement on what methods are appropriate for measuring oxygen transmission rates (OTR) in a wine context or even what the target OTR should be. Given the high number of reported faults in bottled wines stemming from over- or underoxidation, the ability to measure oxygen transmission through a closure and to ensure that a given closure is delivering the intended oxygen dose is of significant commercial importance for the wine industry as a whole. Our research has two main objectives: to experimentally establish our theories about optimal wine oxidation and to confirm that our closures perform within that range. To accomplish these objectives, wine closures were tested using model wines bottled in clear glass and oxygen ingress was measured by different methods. In addition, a variety of commercial wines fitted with closures of known oxygen permeability were tested for postbottling $SO_2$ decline and other chemical measures. We will present the theory behind our targeted degree of in-bottle wine oxygenation, our methods for validating it, and the results of our research to date in creating a closure that reliably delivers oxygen within that range.

*Funding support:* VinPerfect

**Industry – CONTINUED**

### Successful Yeast and Malolactic Bacteria Coinoculation in Red Wine

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The advantage of inducing malolactic fermentation (MLF) by inoculation with selected strains of lactic acid bacteria is two-fold: first, a positive influence on wine flavor and quality and, second, better control over the length and speed of malic acid conversion. Inoculation of wine with malolactic starter cultures is traditionally practiced after the end of the alcoholic fermentation (post-AF), when all fermentable sugars have been consumed by yeast and residual sugars are <2 g/L, to avoid possible production of acetic acid and D-lactic acid, or *picûre lactique*. Inoculation of bacteria starters in the must together with or shortly after yeast inoculation (yeast/bacterial coinoculation) has been proposed as an alternative technique in low pH wines that offers advantages over post-AF inoculation without increasing volatile acidity. Several enological research institutes have recently studied the risks and the benefits of early inoculation with selected malolactic bacteria starter cultures. Under difficult, high-alcohol conditions, inoculation into grape must allowed the bacteria to gradually adapt to the ethanol before it became toxic. This resulted in a clearly reduced total length of malolactic fermentation by the simultaneous inoculation of bacteria with yeast compared to post-AF inoculation and spontaneous MLF. Under easier, high pH conditions, coinoculating the must with yeast and malolactic bacteria allowed early dominance of the selected malolactic bacteria strain, not only over indigenous lactic acid bacteria but also limiting the risk of developing *Brettanomyces* yeast. Using appropriate yeast and malolactic bacteria combinations and applying optimal manufacturing practices, simultaneous inoculation of bacteria with yeast did not affect yeast alcohol fermentation kinetics and no significant difference in volatile acidity was found.

*Funding support:* Lallemand Inc.

### 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 untargeted and targeted screening in one analysis. The objective of this contribution is to demonstrate that given its extreme reproducibility, NMR can detect small changes in

**Industry – CONTINUED**

concentrations of many components in a mixture, which are best monitored by statistical evaluation but which also deliver reliable quantified 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 at most 15 minutes and delivers a multitude of results, which are automatically summarized. The method has been proven on fruit juices, where unknown frauds can be detected, such as addition of sugars and amino acids to fake 100% fruit content, mixing of different varieties, or incorrect product labeling of geographic origin or direct juice versus rediluted concentrate. The methodology developed is now transferred to wine quality control. The advantage of NMR is its reproducibility and transferability from instrument to instrument. Standard operating procedures are required before performing targeted analysis (quantification) on a multitude of organic ingredients (currently >30 are envisaged) or untargeted analysis to generate information such as grape variety, geographic origin, vintage year, mixing of varieties, and unexpected/unknown additives. For untargeted screening, authentic normal models for the individual parameters have to be established, which represent the normal variation of the wines under consideration. Once this is achieved, uni- 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

## Strain Identification of *Saccharomyces cerevisiae* by DNA Fingerprinting

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The wine industry has expressed an interest in identifying individual strains of *Saccharomyces cerevisiae* present in fermentations. Research over the past 20 years has shown that DNA fingerprinting using PCR primers to amplify variable number tandem repeat (VNTR) markers is an effective method to discriminate among yeast strains. However, a commercially available tool using VNTR markers was not available to the industry in the United States. To meet this industry need, ETS has developed a multiplex VNTR analysis method that uses five loci, providing sufficient resolution to track population changes that can occur during fermentation. VNTR marker sizes proved stable after five consecutive reinoculation cycles without divergence from the starting strain isolate. With our database containing over 80 different commercial yeast isolates, we can discriminate among most strains,

**Industry – CONTINUED**

providing positive identification of commonly encountered commercial and indigenous strains. ETS VNTR analysis has been used successfully to monitor yeast population dynamics in native fermentations and to isolate unique strains. The analysis can also be used as a quality control tool to determine the efficacy of inoculations with commercial strains and verify that desired strains are dominating individual fermentations. Future yeast strain selections can now be tailored to the unique conditions of individual wineries.

*Funding support:* ETS Laboratories

## Polyphenol Extraction Management

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In today's winemaking, managing the extraction of polyphenols during fermentation of red varietals becomes more and more challenging: changes in weather patterns, yield management, evolution of wine styles (riper, bolder, bigger wines), production cost reductions, and time management make necessary a management of extraction that has become one of the most important steps in reaching a wine profile objective. Before examining extraction, we studied the evolution of maturity in the vineyard weekly, from veraison to harvest, by measuring the total phenols and anthocyanins and the kinetics of sugar loading. This enabled us to define two main types of maturity: fresh fruit and ripe fruit. We used Merlot harvested from a single vineyard in the Central Coast and applied eight distinct extraction protocols. Two protocols were applied at a ripe fruit maturity and six were applied at a fresh fruit maturity. Each maturity had a control protocol for comparison. Throughout the fermentation, polyphenols were analyzed daily with the same method, using a spectrophotometer. A sensory analysis of preferences with defined descriptors was done at three times: after alcoholic fermentation, after microoxygenation, and after malolactic fermentation. All sensory analysis was done blind by a panel of at least five winemakers and treated statistically. After the importance of maturity management, the control and understanding of polyphenol extractability is a critical point in the winemaking process. It is, then, essential to combine vineyard and winery work. The goal is to apply a specific extraction protocol at each type of maturity to reach the targeted wine profile.

*Funding support:* Vivelys USA

## Industry – CONTINUED

### Tartrate Stability beyond White Wines: Winery Application of Electrodialysis on Red Wines

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A method of continuous tartrate stability using electrodialysis (i.e., STARS) is evaluated. In comparison to traditional bulk refrigeration or cold stabilization, this method offers potential advantages, such as elimination of color and phenolic loss, wine loss, need for seeding, faster stabilization speed, reduced wine handling, and elimination of repeat stability checks. A new method in predicting electrodialysis treatment rate via STABILAB (DIT: degree of tartrate instability) allows precise and batch-specific application of electrodialysis, resulting in minimal pH shift and sensory impact. Data on the financial, wine profile, and sensory implications of the new method are presented.

*Funding support:* Oenodia

### Velocity Winemaking–Continuous Microbial and Tartaric Stabilization: Winery Application of Cross-Flow Microfiltration Coupled with Electrodialysis

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A method of continuous stability is evaluated using ceramic cross-flow microfiltration coupled with electrodialysis, or MEMBSTAB. In comparison to traditional bulk refrigeration or cold stabilization followed by filtration, this method offers potential advantages such as elimination of color and phenolic loss, wine loss, and the need for seeding or diatomaceous earth. The method also gives an overall faster processing speed (schedule control), reduces wine handling, and eliminates repeat stability checks. Combining cross-flow microfiltration's advantages with a new method in predicting electrodialysis treatment rate via STABILAB (DIT: degree of tartrate instability) allows for precise and batch-specific application of electrodialysis, resulting in minimal pH shift and sensory impact. This application can be coupled with existing centrifugal technology for heat stability to further optimize wine stabilization. Data on the financial, wine profile, and sensory implications of the new method are presented.

*Funding support:* Oenodia

**Industry – CONTINUED**

## Allergen-Free Fining Agents: Alternative Fining Materials to Egg Albumin and Casein/Potassium Caseinate

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The growing recognition of food allergies and intolerances, from which many individuals suffer, has led to revision of legislation controlling the labeling of food products. European Directive 2000/13/CE defines substances that are recognized as the source of the most common forms of food allergies and intolerances. The presence of these substances must be declared on the label when they are found in food as ingredients, additives, ingredients of ingredients, additives of ingredients, or technological coadjuncts. Casein/potassium caseinate and egg albumin are commonly used fining agents in wine, and the recent changes in regulation will impact winemaking practices. Allergen-free, pure pea protein and blends with other fining materials like PVPP, silica, or bentonite were compared with traditional fining agents containing casein or potassium caseinate. Also, high molecular weight pork gelatins (not considered allergens) were tested in comparison to egg albumin. Wines treated with potassium caseinate and its allergen-free alternatives were evaluated for efficacy in removing phenolic substances, in particular those involved in oxidation, reducing bitterness, the ability to form complexes with copper and iron, and removing off flavors. Total phenols (OD 280 nm), yellow color (OD 420), pinking potential, and catechin were measured in wines treated with the different fining agents. Pure pea protein has a higher clarifying effect than potassium caseinate-based fining agents, and pea protein blends with other fining agents (PVPP or bentonite) have similar effects with regard to phenols removal. Wines treated with egg albumin and gelatins were compared for clarification and reduction of astringency. Gelatins with high molecular weights have given similar, and often even better, results than egg albumin. Pea protein-based fining agents are proven effective alternatives to potassium caseinate and casein, and high molecular weight gelatins will allow winemakers to obtain the same or better results than if using egg albumin. This work demonstrates that winemakers have effective alternatives that allow them to avoid allergen labeling.

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