

# Abstracts

## Papers and Posters Presented at the ASEV 56th Annual Meeting 22–24 June 2005, Seattle, Washington

### Enology Oral Session, Wednesday

#### Quantitation of Tannins in Australian Grape and Wine Samples: Application of a Simple and Robust Tannin Assay

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Tannins play a vital role for color and mouthfeel in red wine. The ability to adjust the tannin profile of red wine to a particular style or market would be aided by a robust and effective method of tannin measurement. While a number of methods for tannin quantification have been published, very few have been implemented in the wine industry, most likely because of their complexity and lack of specificity. Therefore, the primary aim of this project was to design a simple, robust, and inexpensive tannin assay that could be easily implemented by practitioners in the wine industry. Tannins are water-soluble phenolics with the ability to precipitate proteins and other polymers. An assay using a hydrophobic, commercially available polymer for precipitation of tannin was optimized for use with red wine and 50% ethanol grape homogenate extract. The polymer, which has almost no absorbance at 280 nm, allows for direct spectral measurement of tannin at 280 nm without the need for indirect colorimetric detection. Furthermore, the assay response was shown to correlate with the sensory perception of red wine when increasing amounts of grape-seed derived tannin were added. The assay enables the complete precipitation of tannin from red wine and 50% ethanol grape homogenate extracts and does not suffer interference by other 280 nm absorbing phenolics such as anthocyanins and flavanoids.

#### Polymerization of Anthocyanins with Tannins in Young Norton Wine

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Preliminary studies have found Norton (*Vitis aestivalis*) wines to be relatively high in free anthocyanins but low in tannins and polymeric anthocyanins. These wines often show little of the polymerization of anthocyanins with tannins that is necessary for the stabilization of red wine color. One of the variables thought to influence the formation of polymerized

anthocyanins is the anthocyanin/tannin ratio. A factorial experiment (three levels of tannin addition and three levels of grape skins) was conducted to study the effects of varying the anthocyanin/tannin ratio on the formation of polymeric anthocyanins in a Norton wine. To vary the anthocyanin concentrations, the musts were fermented (2-L flasks) using three different amounts of grape skins (one-third, two-thirds, and three-thirds of the normal content) while retaining the original seed levels. To vary tannin concentrations, three different levels of commercial enological tannin (0, 400, or 800 mg/L) were added to the musts. The 400 mg/L tannin additions were made at the start of fermentation. The 800 mg/L tannin additions were split into 400 mg/L at the start of fermentation and 400 mg/L the day after pressing. The wines were fermented on the skins and seeds for five days. Enological tannin additions had no significant effects on polymeric anthocyanin levels. Reducing the amount of skins present during fermentation resulted in lower levels of polymeric anthocyanins. Polymeric phenols increased by adding tannin and by fermenting with a higher percentage of skins.

#### Modeling Anaerobic Aging of Red Wine

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The research focused on the formation of polymeric pigment in red wine. The goal of the research was to enable one to be able to predict the final amount of polymeric pigment formed based on the initial conditions as measured by the Adams assay. While the research presents multiple different models depending on how one wants to measure polymeric pigment, the results were fairly constant across the models. The amount of polymeric pigment formed seemed to be determined by the amount of tannin as measured as phenolics in the Adams assay instead of anthocyanin. This means that winemakers who try to increase the initial color of the wine may not in fact be affecting the long-term color of the wine. Another potentially significant finding is that Zinfandel seems to form polymeric pigment in a different manner than other red wines. Most of the models used to predict polymeric pigment required a Zinfandel dummy variable term to predict final wine color with a high  $R^2$  value.

#### Effect of Oxygenation Dosage Rate on the Chemical and Sensory Properties of Cabernet Sauvignon

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Controlled oxygenation between the fermentation and bottling stages has become an increasingly popular method of improving sensory characteristics for many red wines. Anecdotal, the success of the treatment appears to be highly

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Abstracts for session presentations are in the approximate order as noted in the conference program. Abstracts here are those submitted and accepted through the 2005 Call for Papers.

\*Indicates corresponding author. **Bold type** indicate presenting author.

dependent on the rate of oxygen delivery. A small-scale, fully replicated trial was conducted to assess the chemical and organoleptic transformations of a Cabernet Sauvignon wine exposed to a range of four oxygen dosage rates (zero, low, medium, and high). Accurate oxygenation of small volumes was achieved using diffusion through a dense polymer membrane, a technique developed at the University of Auckland. Sensory changes were quantified using a trained panel and quantitative descriptive analysis methods. Techniques such as reversed-phase HPLC and thiolysis were employed to provide analysis of the wine polyphenols. Acetaldehyde was also analyzed using GC and color measurements using spectrophotometry. Dissolved oxygen measurements were taken on a weekly basis to track the amount of residual oxygen remaining in the wine. Sensory and chemical analyses were conducted on each treatment rate every two weeks for the duration of the trial (15 weeks). The evolution of the wine was found to depend significantly on the oxygen dosage rate applied, reflected in both the sensory and chemical changes observed. There appears, therefore, to be an optimum or critical rate essential for achieving desired sensory improvements.

### Interactions of Different Mixed Yeast Starter Cultures with *Dekkera bruxellensis* in Wine

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The objective of this study was to investigate whether the use of non-*Saccharomyces* yeasts in defined mixed yeast starter cultures affected the presence of the spoilage yeast *Dekkera bruxellensis* in wine. The cultures investigated contained *Saccharomyces cerevisiae* mixed with *Kluyveromyces thermotolerans* and/or *Torulaspota delbrueckii*. As a control, a pure *S. cerevisiae* culture was used. Growth experiments with *D. bruxellensis* were conducted in supernatants of a commercial grape juice, following alcoholic fermentation with the mixed cultures and the control. All experimental steps, including the alcoholic fermentation, were performed at 25°C. *Dekkera bruxellensis* was inoculated at a low level ( $10^3$  cfu/mL) and its presence was followed by plate counting on a selective agar medium. It was able to grow from the initial concentration of  $10^3$  cfu/mL to a final concentration of  $\sim 10^7$  cfu/mL within 10 days in all supernatants. Growth of *D. bruxellensis* in supernatants from mixed cultures of *K. thermotolerans/S. cerevisiae*, *T. delbrueckii/S. cerevisiae*, and *K. thermotolerans/T. delbrueckii/S. cerevisiae* was similar to its growth in the control supernatant. Thus, the use of *K. thermotolerans* and *T. delbrueckii* in mixed starter cultures with *S. cerevisiae* neither inhibited nor stimulated the presence of *D. bruxellensis* in wine as compared with the use of pure *S. cerevisiae* starter cultures.

### Volatile Phenols Off-Odor Production by Different Strains of *Brettanomyces bruxellensis*

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Volatile phenol off-odors are caused by very potent odors produced by some yeasts and molds from hydroxy-

cinnamic acid precursors. In particular, we are interested in the ethyl phenols produced from *p*-coumaric, caffeic, and ferulic acid. These phenolic acids are natural compounds of wine, cider, beer, and some fruit-juice based beverages. Leather, barnyard, and horse sweat are often used to describe the off-flavors related to ethyl phenols. By decarboxylation of phenolic acids, the correspondent vinyl phenols are produced. By reduction, these vinyl phenols then can be converted to the correspondent ethyl phenols. *Brettanomyces bruxellensis* is better studied among the yeasts producing ethyl phenols. *Brettanomyces* has long been recognized as a widespread contaminant of wine. *Brettanomyces bruxellensis* and *anomalus* are the only species among *Brettanomyces* capable of producing ethyl phenols. The strains used in the experiment previously showed a difference in ethyl phenol production. We related these differences to different volatile phenol activity among strains and studied this activity under various conditions. Volatile polyphenol formation was followed spectrophotometrically. In particular we studied a strain incapable of degrading all the *p*-coumaric acid. Indeed *p*-coumaric acid was only partially degraded, and it was possible to observe an accumulation of vinyl phenol. In this study we tried to better understand the yeast metabolism involved in the production of volatile phenol off-flavors.

### Viticulture Oral Session, Wednesday

### Progress toward Breeding Pierce's Disease Resistant Winegrapes

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The Walker lab has been breeding Pierce's disease (PD) resistant grapes for six years. The overall goal is to incorporate a broad range of PD-resistance sources into high-quality *Vitis vinifera* backgrounds. Our first objective was to produce winegrape cultivars suitable for blending and to be used in PD hot spots. About 2,000 seedlings are planted in the field each year. These seedlings are evaluated for high-quality fruit and then screened for *Xylella fastidiosa* (Xf) resistance. This screening is very severe, and material that passes the screening dramatically restricts Xf movement from the inoculation point. Promising, highly resistant selections with good juice evaluations include 0028-35 (neutral flavor, white, good acidity and pH, high juice yields); 0078-02 (light muscat flavor, white, excellent acidity and pH for warm climates); and 0058-23 (dark purple, fruity aromas, low acidity, very productive). Female flowered selections have been made to expedite breeding. These include 0110-050, with dark red berry color, excellent fruit quality, and resistance from DC1-39, and 0028-44, with dark red color, excellent fruit quality, and resistance from Midsouth. Over 300 seedlings are expected to fruit this year from crosses of F2-7 and F2-35 (Ruby Cabernet siblings) x BD5-117, a complex hybrid with multiple resistance sources that has produced greater than 50% resistant progeny in the past. The breeding program is the foundation for genetic mapping efforts from which genetic markers for resistance are developed and resistance genes are characterized.

## Environmental Variables Influencing the Severity of Pierce's Disease and Its Sharpshooter Vectors

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Our general objective was to study and identify the various factors involved in the pathogenicity of Pierce's disease (PD) in grapes, caused by the bacterium *Xylella fastidiosa* (Xf). Our project goal was to rank a set of variables that correspond to an outbreak of disease, which may lead to better management strategies for the disease. By comparing vineyards with high PD incidence to those with little or no problem, with study sites in northern and southern California, we hoped to gain an understanding into possible routes of control for disease. Using a multivariate direct gradient ordination technique (canonical correspondence analysis [CANOCO]), climatic and environmental variables that contribute to increased disease incidence were ranked. Variables in the analysis included soil physical and chemical characteristics, vine nutrition status, vine water status, soil moisture, sharpshooter abundance, alternate vegetation, and climatic extremes. Overall, CANOCO analysis indicated that the most important variable corresponding to the incidence of PD was soil moisture, i.e., the higher the soil moisture, the higher the PD incidence, followed by soil sulfur, vine iron, and vine phosphorus. Variables with the least correspondence included soil iron, vine manganese, and soil cation exchange capacity.

## Pierce's Disease in Texas: Evaluating a PD Hot Zone, Investigating Insect Frequencies, and *Xylella fastidiosa* Genetics

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The temperate Gulf Coast has long been a region of high Pierce's disease pressure. New evidence also suggests the glassy-winged sharpshooter is likely native to Texas. This makes Texas an ideal location to investigate the ecology and epidemiology of a disease that is endemic and not in outbreak mode. Evaluation of a native hybrid vineyard in the Gulf area has shown that many native plants can serve as reservoirs for bacterial levels and that not all hybrid varieties may be equally tolerant. Initial analysis of common insects from eight Texas vineyards shows there are at least three insect species with high frequencies and bacterial levels that may vector the disease in Texas. Differences in insect number also seem to increase with some environmental variables such as reduced weed control. Evaluation of the genetics of *Xylella fastidiosa* strains from grapevines and native plants suggests there is significant variability of *X. fastidiosa* within Texas.

## Differences in Xylem Sap Composition in PD-Tolerant and PD-Susceptible Grape Cultivars

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Pierce's disease (PD) of grapevine has a great impact on bunch grape production in the southern United States. In June

2000, the USDA declared PD an agricultural emergency. PD can kill a grapevine in two years and there is no known cure. All *vinifera* varieties are susceptible to PD, but they vary markedly in levels of tolerance. The bacteria spread more slowly in more tolerant varieties than in more susceptible varieties. In an attempt to understand the basis of such tolerance, the xylem sap composition of several grape genotypes varying in PD-tolerance was studied. Xylem sap was collected from 13 muscadine and 13 California bunch grapevines. The concentration of amino acids and soluble sugars was estimated in the xylem sap. The amount of amino acids and soluble sugars was found to be higher in the California bunch sap when compared to the muscadine sap. The availability of higher nutrients in the sap of California bunch grapevines may be the reason for increased susceptibility to infection by *Xylella fastidiosa*. Separation of xylem sap proteins on tris-tricine SDS-PAGE showed the presence of a unique band in PD-tolerant grapevines.

## A Protocol for Using a Mite Brushing Machine for Estimating Densities of Willamette Mite on Grapes

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Spider mites (family Tetranychidae) are among the most widespread and troublesome grape arthropod pests in California. They feed on leaves with their piercing/sucking mouthparts, destroying leaf tissue and causing a loss of photosynthetically active leaf area. Willamette mite is the dominant mite pest in the winegrape growing regions of the Sierra foothills and the North and Central Coasts. Enumerative methods for estimating field populations of mites can be accurate and precise but time-consuming. Mite brushing is a technology that can reduce the time required to obtain either absolute counts or estimates of arthropods on leaves from samples. Although this technology was investigated decades ago and recommended as a useful tool for research and commercial pest management by research entomologists and the USDA, there has been little work to demonstrate the validity of this technique since the 1950s. In this investigation we analyzed the number of brushing passes for removal of all mites from the leaf, the distribution of mites on the collection plate, the accuracy and precision of various scanning methods, and compared mite brushing with direct visual counts. We also compared the accuracy and precision of mite brushing to traditional binomial sampling and analyzed cost-benefits based on the time required for each protocol.

## Resistance to Root-Knot Nematodes (*Meloidogyne* Species) in Grape Rootstock Germplasm

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Development of rootstocks with resistance to aggressive root-knot nematodes (*Meloidogyne* spp.) is a top priority in grapevine breeding. New sources of resistance are needed to address the challenge of these root-knot nematodes that can reproduce on the rootstocks Freedom and Harmony. To identify



additional nematode-resistant grape rootstock germplasm, 14 accessions from the USDA-ARS Plant Genetic Resources Unit grape collection were tested for resistance. Green-growing cuttings of test accessions and controls were rooted in propagation sponges and then cultivated in small pots in a greenhouse. Each seedling was inoculated with approximately 1500 second stage juveniles of Harmony virulent *Meloidogyne* sp. Reproduction was measured by counting the number of stained nematode egg masses visible per root system. Only two accessions, GVIT 699, a *Vitis doaniana* x *V. riparia*, and GVIT 703, a *V. vulpina* x *V. riparia* accession, completely suppressed nematode reproduction. *Vitis acerifolia* accessions tended to demonstrate high levels of resistance, although none completely suppressed nematode reproduction. The highly resistant accessions identified in this study are potential sources of nematode resistance for rootstock breeding and some might be useful for direct rootstock use.

### Association of Weed Species with Vineyard Floor Management Practices in California

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A long-term, multidisciplinary trial evaluating the impact of various vineyard floor management practices on weeds was initiated in 2001 in Greenfield, CA. Using a split-block design, three weed-control strategies were assigned to the vine rows: (1) preemergence, simazine + oxyfluorfen, followed by post-emergence, glyphosate + oxyfluorfen; (2) postemergence only, glyphosate + oxyfluorfen; and (3) cultivation only, Clemens. Three cover-crop strategies were assigned to the row middles: bare control, Merced rye, and Trios 102 triticale. Weed frequency was measured four times each year and evaluation of data over four years indicated that distinct weed communities developed in each weed-control treatment. There were low levels of purslane (*Portulaca oleracea*) before the initiation of the trial; however, over four years, purslane increased dramatically and is the dominant summer weed in the cultivation treatment. Likewise in the postemergence treatment, horseweed (*Conyza canadensis*) increased over the years and is the most common weed. This increase in horseweed may be related to the use of glyphosate and oxyfluorfen, which both provide limited control of this weed and which necessitated an application of glufosinate in each of the past two seasons to bring it under control. Nutsedge (*Cyperus esculentus*) is the most dominant weed in the preemergence weed-control treatment because it is tolerant of the preemergence herbicides; however, it is under partial control with post-emergent applications of glyphosate. Weed species tolerant of each of the weed control treatments became dominant over the four years of the trial.

#### Student Enology Oral Session, Wednesday

### Development of Advanced Technologies for the Prevention of Protein Haze Formation in White Wine

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cal Engineering, University of Adelaide, SA 5005 Australia [email: ccolby@chemeng.adelaide.edu.au]

Protein-induced wine haze is a major concern to the wine industry worldwide. While the presence of protein haze is unlikely to affect sensory profile, consumers often reject wines containing hazes as they appear microbially spoiled. Consequently, an important step during commercial winemaking is to treat wines with bentonite, which removes heat unstable proteins by adsorption and prevents haze formation. While this process is effective, it is claimed to adversely affect the quality of the treated wine under certain conditions and 3 to 10% of the wine volume is typically occluded in bentonite lees. This wine is either lost or substantially diminished in quality and value during recovery. Therefore, alternative and economically viable process technologies that maintain wine quality and reduce costs are highly desirable. This paper presents an overview of current research by the authors into innovative approaches to haze prevention, including inline-dosing of bentonite, protein adsorption using thermally modified bentonites, and combined heat/enzyme treatment of wine. Simulations of these processes are currently being developed for process optimization and comparison with results from laboratory and large-scale field trials. This research, established jointly by the University of Adelaide, the Australian Wine Research Institute, and the Hardy Wine Company, forms part of the GWRDC (Australia) project entitled "Better quality wine and lower production costs from new processing technologies for protein haze removal."

### Characterization of Tannin Levels and Polymeric Pigment Development in Syrah Wines

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In order to evaluate phenolic extraction from Syrah fruit during winemaking, a protein precipitation assay was used to measure tannin, iron-reactive phenolics, polymeric pigment, and monomeric anthocyanin levels in berries at harvest and the corresponding wines at pressing. Ten sets of skin and seed samples from five wineries were collected, extracted, and analyzed. We evaluated fruit at harvest, skin and seed samples from pomace, wines at pressing, and wines after 105 days of barrel aging. To characterize temperature effects on polymeric pigment formation and anthocyanin degradation during aging, we collected samples from 25 commercial Syrah fermentations at pressing. Four subsamples were created from each lot and one aliquot from each was placed into one of four different temperature control rooms maintained at 5, 10, 20, and 28°C. These samples were analyzed periodically for 105 days, and the 105-day samples were compared with 105-day samples provided by the winery from their barrels or tanks. The temperature-controlled samples had mean monomeric anthocyanin losses of 22, 34, 47, and 74%, respectively. To characterize tannin and phenolic levels in commercial Syrah wines, we analyzed 250 finished and commercially available Syrah wines from California, Oregon, Washington, and Australia using the same phenolics panel as described above. The mean tannin level was 491 mg/L catechin equivalents and the range was from 78 to 999 mg/L.

## Phenolics in Fruit and Wines of Pinot noir from the Anderson Valley

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In a previous survey of Pinot noir wines from AVAs in California and Oregon, wines from Anderson Valley were found to have the second lowest mean tannin level of any of the AVAs surveyed. To determine if this could be due to fruit composition or extraction phenomena, a study was undertaken with Anderson Valley fruit to characterize phenolic levels in berries at harvest, in the corresponding wines at pressing, and after 120 days of barrel aging. A protein precipitation assay was used to measure tannin, polymeric pigment, iron-reactive phenolics, and monomeric anthocyanins in fruit and wines from Anderson Valley. Six lots of Dijon 115 clone and five lots of Dijon 667 clone from different vineyards within the Anderson Valley were examined. For phenolic analysis, skin and seed samples were collected from the fruit at harvest, from the pomace from each lot after pressing, and from the corresponding wines at pressing and after 120 days of aging. We also assayed 41 commercial wines at pressing and again after 120 days of barrel aging. The average anthocyanin level in the 41 wines at pressing was 429 mg/L malvidin-3-glucoside equivalents (M3GE) with a range of 229 to 585 mg/L. After approximately 120 days of barrel aging, the wines had an average of 224 mg/L M3GE with a range of 112 to 357 mg/L. Thus, during barrel aging, a dramatic drop occurred in anthocyanins averaging 48% with a concomitant increase in polymeric pigments. The half life of the monomeric anthocyanins was found to be similar across all lots examined.

## Characterization of Wine Pigments from Different Fractions Obtained by Countercurrent Chromatography

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Color changes in wine are due to gradual conversion of the native anthocyanin pigments extracted from red grape into various anthocyanin-derived pigments through different reaction mechanisms that occur during winemaking and aging. Fractionation and eventually isolation of these pigments are fundamental to determine their chemical and color properties and to compare them to those of native anthocyanins. A red wine, made from Cabernet Sauvignon (60%) and Tannat (40%) cultivars, was fractionated by high-speed countercurrent chromatography (HSCCC). The solvent system consisting of *tert*-butyl methyl ether/*n*-butanol/acetonitrile/water (2/2/1/5, acidified with 0.1% TFA) was chosen for its demonstrated efficiency in separating anthocyanins. Five different fractions and the organic stationary phase were collected and analyzed. The different native and derived anthocyanins were identified on the basis of their UV-visible spectra, their elution time on reversed-phase HPLC, and their mass spectra, before and after thiolysis. The HSCCC method allowed the separation of different families of anthocyanin-derived pigments that were eluted in different fractions according to their structures. The hydrosoluble fraction (fraction 1) was almost devoid of na-

tive anthocyanins. Further characterization of fraction 1 (glucose quantification, UV-visible absorbance measurements) indicated that it contained flavanol and anthocyanin copolymers in which part of the anthocyanin units were in colorless forms. Pigments in the hydrosoluble fraction showed increased resistance to sulfite bleaching and to the nucleophilic attack of water.

## Continuous Temperature Monitoring in a Cabernet Sauvignon Fermentation with a Custom-Designed Temperature Monitoring Cable

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Many important processes in wine production are dramatically influenced by temperature. Temperature gradients form naturally during fermentation because of the metabolic activities of yeast and differential rates of heat exchange. By understanding temperature gradient formation in wine fermentations and the effect these have on wine quality, winemakers can formulate better process-control decisions for a given batch of grapes. In this experiment, temperatures were monitored continuously during a Cabernet Sauvignon fermentation (2000 L) using a custom-designed temperature monitoring cable. The 24-sensor cable was installed vertically with 12 sensors positioned through the center of the tank and 12 along the inside wall so that both horizontal and vertical differentials were determinable. Automated pump-overs occurred every 12 hours (1 tank volume in 20 min). Vertical temperature differentials were greatest during the most active fermentation phase, and temperature differences as high as 7 C were seen between the cap and fermenting juice. Heat build-up in the cap was distributed throughout the tank in the first five minutes of the pump-overs. The time required to reestablish the temperature gradient in the tank was dependent on the fermentation rate. Horizontal temperature differences were much less dramatic (0 to 3 C) and were moderated by pump-overs and heat exchange between the tank and winery air. Tank samples taken throughout the fermentation were analyzed for polymeric pigment, tannins, anthocyanins, and total phenols.

## Impact of Low Doses of Oxygen Addition on Wine Phenolic Constituents

**Felipe Laurie**, Robert Law, Willy S. Joslin, and Andrew L. Waterhouse\*. Department of Viticulture and Enology, University of California, Davis, CA 95616 [email: alwaterhouse@ucdavis.edu]

The contribution of oxygen at low doses to the color stability, aging chemistry, and flavor of wine is a complex and still unresolved matter. Contrary to previous claims, results of recent research gathered by this group have established that low-oxygen addition (microoxygenation) augments the level of dissolved oxygen (DO) in wine but that it varies in its effect on phenolic concentration and composition. In this experiment, different regimes of low-level oxygenation and oxygen-delivery systems were investigated on a commercial scale. Cabernet Sauvignon in pre- and postmalolactic fermentation was treated with oxygen at variable flow rates (1.0 to

60 mL of oxygen/L of wine/month) and analyzed with sensitive methods for DO (in-line), acetaldehyde, pyrazines, and phenolics. Considering the capacity of wine to absorb large amounts of oxygen, it was surprising that doses as low as 1.0 mL/L of wine/month increased the level of DO regardless of the oxygen delivery system used. No differences in acetaldehyde or 3-iso-butyl-methoxy-pyrazine were observed, but increments in phenolic polymerization were detected. These results confirmed that chemical reactions of oxygen in wine are very slow and complex and suggest the necessity for more fundamental studies to elucidate the phenol oxidative mechanisms.

### Student Viticulture Oral Session, Wednesday

#### Cell Viability as Influenced by Hang Time and Berry Shriveling in Cabernet Sauvignon Fruit

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Fluorescein diacetate, a stain commonly used to evaluate pollen viability, was used to test the hypothesis that cell viability and membrane integrity declines in grape berry cells at veraison as part of normal berry development. A fluorescein diacetate solution was applied to longitudinal hand sections of berries at various ages. Viable mesocarp cells with intact membranes showed strong cytoplasmic fluorescence, which appeared as an outline on the periphery of the cell. This pattern of fluorescence was observed under both the dissecting microscope and confocal laser scanning microscopy. Field samples of Cabernet Sauvignon berries were collected approximately weekly 30 to 160 days after anthesis (DAA). In normally developing berries, veraison occurred at 56 DAA (8.5 Brix), and from 30 to 140 DAA (4 to 27 Brix) there was essentially no loss of mesocarp cell viability. On the final sampling date (154 DAA), there was about 35% loss in viability. For fruit exhibiting berry shrivel, a disorder affecting sugar accumulation and berry maturation, a clear loss of viability in the inner mesocarp was observed starting at approximately 70 DAA (16 Brix), and by the final sample date there was about 76% loss in viability. We conclude that normal grape berry development does not involve a widespread loss of membrane integrity around veraison as previously hypothesized and that cells remain viable far into berry development. Based on this evidence it is also likely the berries remain metabolically active, retaining the ability to accumulate sugars, pigments, and other compounds that are important components of wine quality.

#### Abscisic Acid Concentration and Timing for Color Improvement of Table Grapes

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Poor color development is a major problem in many California tablegrape (*Vitis vinifera* L.) vineyards, reducing fruit quality and lowering packable yields. Application of abscisic acid (ABA) to grape berries may increase the anthocyanin content of their skins, but until recently the high cost of

ABA prohibited commercial application. However, a process was recently developed whereby ABA can be produced much more economically, enabling commercial application. Experiments were conducted in 2003 and 2004 to determine the ABA application times and concentrations that were most effective at increasing peel anthocyanin levels of Crimson Seedless, Flame Seedless, and Red Globe berries. In 2003, veraison was the best application time, regardless of cultivar. The relatively high rates tested in 2003 (500 and 1,000 mg/L ABA) resulted in excessive berry color; lower rates (0, 75, 150, and 300 mg/L ABA) were tested in 2004. Application of ABA at or after veraison (4 to 8 weeks after) markedly increased anthocyanin concentration of Crimson Seedless berry skins when harvested in late September or early October. The most effective rate was 300 mg/L ABA. Similarly, application of 150 to 300 mg/L ABA at veraison, or a few weeks after, increased anthocyanins of Flame Seedless more than 30%, for fruit harvested between mid- and late July. Abscisic acid was ineffective at enhancing anthocyanin concentration of Red Globe grapes. Additional data are needed to confirm these observations, especially considering that unusual weather conditions in 2004 resulted in early fruit maturity and good color development of all grapes tested.

#### Using Near Infrared Spectroscopy as an Analytical Tool in Vineyards and Wineries

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Near infrared (NIR) spectroscopy is a powerful analytical tool for the grape and wine industries. A portable NIR spectrometer was used to rapidly and nondestructively analyze nine chemical characteristics of winegrapes. In 2003 and 2004, over 7,000 NIR spectra (1100 to 2300 nm) were collected from 1,500 fruit samples, using a variety of grape cultivars at various stages of maturity. These samples were then analyzed by standard reference methodologies. Correlations between NIR and reference data were determined by chemo-statistical software. Calibration models derived from this data yield the following correlation coefficients: Brix (.91), pH (.75), TA (.76), tartaric acid (.75), malic acid (.80), NH<sub>3</sub> (.73), amino nitrogen (.73), potassium (.83), and anthocyanins (.82). Current data suggests that these correlations may be improved by grouping cultivars of similar tissue structures and by compensating for variability in fruit temperature and berry turgidity. Subsets within the data have shown differences between grapes at various stages of maturity, as well as grapes subjected to different growing conditions. Interpretation of these spectral differences is likely to provide quantitative measurements to evaluate the effects of viticultural practices. The same NIR spectrometer can be used with a liquid probe to evaluate must and clarified wines. Calibrations are currently being developed to quantify EtOH, SO<sub>2</sub>, color, and total phenolics in wine.



### **Cultural and Processing Impacts on the Relationship between Clone and Growing Location on Anthocyanin Profile of Pinot noir**

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The improved culture and vinification of *Vitis vinifera* L. Pinot noir is a highly topical issue throughout the wine world. The quality of the final product and the color issues associated with cooler growing climates are significantly altered across clone and location of the vines. There is an increased requirement for improved practices in both the vineyard and the winery to establish optimum production criteria for individual combinations of clone and location. Over a two-season period in Michigan and Canterbury, New Zealand, established vines trained to a two-cane vertical shoot-positioning system were selected and treated uniformly with structured canopy management techniques. Clusters were sampled at regular intervals from veraison to harvest and processed for HPLC analysis. Alcoholic fermentation was carried out on remaining fruit using isolated processing methods, which also led to HPLC analysis. Data suggest clone and location impact anthocyanin profiles for *V. vinifera* L. Pinot noir.

### **Predicting the Influence of Viticultural Practices on Phenolic Profile, Color, and Astringency of Napa Valley Cabernet Sauvignon Wines for Precision Viticulture**

**Hua Chen**, Hildegard Heymann, and David E. Block\*. Department of Viticulture and Enology, University of California, Davis, CA 95616 [email: deblock@ucdavis.edu]

To reveal the relationship between simultaneous multiple viticultural practices and the sensory and chemical characteristics of final wines, 12 viticultural practices (such as rootstock, vine density, pruning level) were varied using existing Cabernet Sauvignon viticultural trials at the Oakville Experimental Vineyards (CA) over three vintages from 2000 to 2002. In all, 38 combinations of treatments were chosen each year. The grapes from each lot were identically processed into final wines at the UC Davis research winery. Descriptive sensory panel and chemical analysis (such as pH, TA, HPLC, reversed-phase HPLC, Harbertson-Adams assay and copigmentation assay) were conducted on these wines. Data were analyzed by multivariate statistical analysis to disclose the interrelationship among the phenolic profile, color, astringency, and sourness of the wines. General regression neural networks and other database mining methods were then used to identify the viticultural practices critical in determining the chemical characteristics and astringency of the wines. Models were formed in order to predict the effects of these critical practices on final wine quality. Case studies demonstrate how this type of model can be used by vineyard managers to predict optimal sets of manipulable viticultural treatments to achieve wines with specified sensory and chemical characteristics.

### **Influence of Vine-Vigor Variation within Vineyard on Pinot noir Grape and Wine Proanthocyanidins**

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The research goal was to investigate how variability in soil/landscape characteristics within a commercial vineyard influenced wine quality with an emphasis on phenolic chemistry. The specific hypothesis was that zones within the vineyard with reduced vine vigor would produce fruit with higher concentrations of proanthocyanidins in the fruit and wine. A practical application was to determine the technical feasibility of using precision agriculture tools to manage wine style in cool-climate vineyards. The study was done in two commercial vineyards where blocks within each vineyard consisted of the same clone, rootstock, age, and vineyard management practices. The experimental design involved monitoring soil, vine growth, yield components, and fruit composition on a grid pattern by GPS location to assess patterns in growth and development. Vine-vigor parameters were used to designate zones within both blocks to produce research wines to investigate the vine-fruit-wine continuum. There was no significant influence of vine vigor on proanthocyanidin per seed and only minimal differences in seed proanthocyanidin composition. The major findings were a large increase in skin proanthocyanidin (mg/berry), proportion of (-)-epigallocatechin, molecular mass, and pigmented polymer content in fruit from zones with decreasing vine vigor. In the wines produced from low-vigor zones, there were increases in proanthocyanidin concentration in general and skin proanthocyanidin concentration in particular. In addition, an increase in proanthocyanidin molecular mass and pigmented polymers was observed, while the flavan-3-ol monomer concentration decreased.

#### **Student Enology Oral Session, Thursday**

### **Reduction of Wine Acetaldehyde Levels by Lactic Acid Bacteria**

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Acetaldehyde is the most important carbonyl compound produced during alcoholic fermentation. It is highly relevant for red wine color development and has a distinctive aroma, which in most table wines is masked by the addition of sulfur dioxide. However, acetaldehyde-bound sulfur dioxide loses its antioxidant and antimicrobial activities, requiring compensation with higher sulfur dioxide additions, which may cause adverse reactions in sensitive consumers. We focused on the ability of lactic acid bacteria to degrade acetaldehyde during malolactic fermentations. In a preliminary study, we surveyed 100 wines for their acetaldehyde concentrations and compared the results with other wine parameters, including wine color, style, variety, and winery. We then studied the growth of lactic acid bacteria under different conditions (pH, acetaldehyde and SO<sub>2</sub> concentrations) and evaluated the bacterial acetaldehyde degradation capacity. Commercially available lactic acid bacteria and isolates from Ontario wines undergoing spontaneous

malolactic fermentations were used. The strains were tested both before and after 5-month growth adaptation to high acetaldehyde concentrations. The survey of Ontario wines revealed that acetaldehyde concentrations were predominantly wine-color and winery specific. Some lactic acid bacteria strains could be adapted to grow in media containing as much as 1.7 g/L acetaldehyde. Comparison of bacterial growth at different acetaldehyde concentrations and the bacterial acetaldehyde degradation capacity revealed strain-specific differences between adapted and nonadapted strains, suggesting applications of strong acetaldehyde degrading bacteria for the acetaldehyde reduction in wine. Growth response of lactic acid bacteria to acetaldehyde was pH- and sulfur dioxide-dependent. This demonstrates that the concentration of bound sulfur dioxide has to be considered in order to evaluate the feasibility of malolactic fermentations.

### Microbial, Chemical, and Sensorial Considerations for Managing High-Density Fermentations

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Commercial volumes of high-density Syrah and Chardonnay were fermented under different temperature levels, nutrient programs, and dilutions to determine their effects on avoiding stuck fermentations. The resulting wines were then compared using a triangle discrimination test to determine any sensory effects on the different treatments. Higher fermentation temperature was found to increase the risk of problem fermentations. Gradual nutrition programs, rather than an initial large nutrition application, seemed to decrease the likelihood of a stuck fermentation. Dilution of high-density musts is a viable method for avoiding stuck fermentations. In some cases, there is little or no effect on the resulting wine sensory attributes.

### Bacterial Causes of Winery Chloroanisole Contamination

**Paula A. Mara** and Linda F. Bisson\*. Department of Viticulture and Enology, University of California, Davis, CA 95616 [email: lfbisson@ucdavis.edu]

Winery-dwelling bacteria have been found to produce musty chloroanisole compounds, including 2,4,6-trichloroanisole (TCA) and 2,4,5,6-tetrachloroanisole, from chlorinated phenolic substrates, including those that can originate from chlorine bleach and chlorophenol-based wood preservative residues. These residues can persist in a cool, dark environment for years after their use has been discontinued. Current winery cellar sanitation and management practices are not likely to prevent colonization of surfaces by filamentous and biofilm-forming microbes. The goals of this study were to identify bacterial sources of TCA in the UC Davis teaching winery and cellar as well as bacteria degrading 2,4,6-trichlorophenol (TCP) for carbon. Fifteen *Streptomyces* isolates were obtained from areas of the UC Davis winery and cellar with musty odors and tested for production of TCA from TCP. Two isolates produced recognizable levels of TCA, as determined by GC-MS. In the second part of this study, 150 isolates from

14 sampling locations were screened for the ability to use TCP as a sole carbon source. Twelve isolates that grow well on TCP were obtained. Differences in whether bacteria convert TCP to TCA or break down TCP to use as a carbon source help to explain why winery-wide chloroanisole taint is present in some wineries but not others.

### Impact of Yeast Conditioning on the Sensory Profile of Vidal Icewine

**Derek Kontkanen**, Gary J. Pickering, Andrew Reynolds, and Debra L. Inglis\*. CCOVI, Department of Biological Sciences, Brock University, St. Catharines, ON L2S 3A1, Canada [email: dinglis@brocku.ca]

Icewine is a dessert wine produced from grapes frozen naturally on the vine, resulting in wines concentrated in aromas and flavors, with ~10% v/v ethanol and more than 200 g/L residual sugar. Although icewines have been produced commercially in Canada since the mid-1980s, studies are needed to investigate fermentation conditions that alter the fermentation kinetics along with the chemical composition and sensory properties of these wines. This study investigated the impact that yeast inoculation methods have on the sensory profile of Vidal icewine by testing four different yeast treatments in triplicate. Commercial yeast K1-V1116 were rehydrated with or without the yeast micronutrient mixture GOFERM and then inoculated into icewine juice at a rate of 0.5 g/L, either directly after rehydration or after a stepwise acclimatization procedure. A full descriptive analysis of the 12 wines found eight aromas and flavors that were significantly different between treatments. Icewines produced by direct inoculation without the micronutrient were described with raisin, butter, and spicy aromas, whereas with the micronutrient they were described as sweet in taste with honey and orange flavors. Icewines produced by stepwise acclimatization without the micronutrient were described with peach and terpene aromas, whereas with the micronutrient they were described with pineapple and alcohol aromas and alcohol and honey flavors. Significant differences in fermentation kinetics and yeast metabolite production (ethanol, acetic acid, and glycerol) were also found among the treatments, indicating that stepwise acclimatization reduced the yeast-adapted stress response while fermenting unfiltered icewine juice.

### Predicting Chardonnay Fermentation Kinetics Based on Juice Composition and Processing Decisions

**Doug Nierman**, Hua Chen, Matthew Coleman, Scott Sisemore, Leigh Meyering, Trevor Phister, David Mills, and David Block\*. Department of Viticulture and Enology, University of California, Davis, CA 95616 [email: deblock@ucdavis.edu]

The inability to predict fermentation kinetics, especially for stuck or sluggish fermentations, is a perplexing and often costly wine industry problem. Predicting fermentation kinetics, prior to the beginning of fermentation, would allow winemakers to allocate time and space requirements more efficiently and in some cases take corrective actions at the juice stage to prevent excessively sluggish or stuck fermentations. The objective of this three-year research study was to determine critical juice parameters and processing decisions that could



adequately predict fermentation kinetics. To this end, over 600 Chardonnay juice samples were gathered from commercial wineries across California spanning the 2001, 2002, and 2003 vintages. These samples were analyzed for a variety of parameters, including organic acids, glucose, fructose, ammonia,  $\alpha$ -amino nitrogen, amino acids, vitamins, metal ions, and microbial ecology. The data for the first two vintages have recently been analyzed by a variety of data-mining techniques, including decision tree analysis, partial least squares analysis, general regression neural networks, and hybrid modeling. Thus far, the processing parameter temperature has proven to be a more important determinant of fermentation kinetics than any juice constituent, but other trends have emerged as well with glucose, specific amino acids, specific vitamins, and YANC being consistently chosen as important juice constituents.

### Yeast Strain and Nitrogen Effects on Volatile Ester Production in Chardonnay Juice Fermentation

**Aaron Miller**, Linda Bisson, and Susan Ebeler\*. Department of Viticulture and Enology, University of California, Davis, CA 95616 [email: seebeler@ucdavis.edu]

Volatile esters are a major by-product in the yeast fermentation of grape juice to wine. These esters are extremely important compounds and can have a great impact on the flavor composition of wines. Seven different commercially available strains of *Saccharomyces cerevisiae* were monitored for their production of seven important volatile esters. Chardonnay juice was aseptically inoculated with each strain of *S. cerevisiae* to create monoculture fermentations, and volatile ester concentrations were determined with GC-FID by sampling the headspace with SPME. Significant differences were observed in the kinetics of ester formation as well as in the maximum and final ester concentrations among the yeast strains. Three of these yeast strains, a low, medium, and high concentration ester producer, were further studied to determine the effects of different types of nitrogen additions on ester production. Chardonnay juice was supplemented with either diammonium phosphate, a common nutrient additive in the wine industry, or amino acids, a precursor to esters, and the ester production was monitored in the same manner. Differences in ester production were related to amino acid utilization patterns.

#### Student Viticulture Oral Session, Thursday

### Systematics of *Vitis*

**Eric Wada**, M. Andrew Walker\*, and Daniel Potter. Department of Viticulture and Enology, University of California, Davis, CA 95616 [email: awalker@ucdavis.edu]

The taxonomy of wild species of *Vitis* has been reputed to be difficult. Previous studies base their varying numbers of species within the genus on morphological characteristics and geographic distribution. The incidence of natural hybridization in the wild is thought to confuse *Vitis* taxonomy. The purpose of our study was to reconstruct phylogenetic relationships within *Vitis* in an attempt to describe species boundaries. Plants in the study were acquired from the UC Davis Viticulture and Enology vineyards, the USDA germplasm repository in Davis, CA, and from wild populations in Texas,

New Mexico, and Arizona. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA and an intergenic spacer of the chloroplast genome were sequenced. Preliminary analysis suggests that speciation is a relatively recent event in *Vitis*. There appear to be at least four species in Arizona, yet only one is listed in taxonomic guides. Although the others (*Vitis arizonica*, *V. treleasei*, *V. acerifolia*, and several hybrid forms with *V. candidans* or *V. champinii*) are present, they were not distinguished with sequencing efforts, thus providing more evidence of their hybrid nature and recent evolution. Many currently recognized species may be considered subspecies due to low sequence variation; some currently recognized species might actually represent hybrids of other species. Finally, the ITS region is potentially useful for identifying recent hybrids in the wild.

### Resistance to *Xiphinema index* in a Population of *Vitis* Species Collected in Northern Mexico

**Jeffrey Wheeler** and Andrew Walker\*. Department of Viticulture and Enology, University of California, Davis, CA 95616 [email: awalker@ucdavis.edu]

A wide range of grape species material from northern and central Mexico was collected by H.P. Olmo in 1961. Previous testing in the Walker lab determined that several of these accessions were inadvertent parents in rootstock selections with excellent resistance to *Xiphinema index*, the dagger nematode vector of grapevine fanleaf virus. The resistant forms appeared to be *Vitis arizonica* and close relatives; however, the grape species from Mexico and the southwest United States are very poorly described. The Walker lab is currently researching this group of species and has delineated many of the accessions collected by Olmo. We hypothesized that resistance to *X. index* should be common in these accessions. Sixty-five genotypes were grown in small pots and inoculated with 100 nematodes. After 8 weeks, their root tips were examined for galling as an indicator of resistance. Fifteen of the genotypes did not produce galls. St. George controls produced 25 galls in the same time period. The greatest percentage of resistant genotypes (five of nine) was found in *V. arizonica* forms that appear like those from southern Arizona and Mexico. Three of six *V. treleasei* forms were resistant. Seven of the 20 tested *V. cinerea* types were resistant. There was no apparent relationship between the collection location and *X. index* resistance.

### Correlating Root System Cytokinin Production with Tolerance to Fanleaf

**Brady Smith**, Kirsten Skogerson, and Andrew Walker\*. Department of Viticulture and Enology, University of California, Davis, CA 95616 [email: awalker@ucdavis.edu]

Grapevine fanleaf virus (GFLV) is vectored by *Xiphinema index*, the dagger nematode, and together they cause a disease complex known as fanleaf degeneration or fanleaf. Fanleaf causes millions of dollars in damage to worldwide grape production because of GFLV's impact on flowering and *X. index*'s feeding damage to the root system. There are rootstocks that prevent *X. index* feeding damage, but they allow transmission of GFLV to the scion. Two *Muscadinia rotundifolia* based rootstocks, O39-16 and O43-43, induce tolerance to GFLV's disruption of fertilization and therefore allow normal seed set

and cluster weights. We hypothesized that a rootstock-generated phytohormone is compensating for GFLV's impact on flowering. The most obvious phytohormone that may generate tolerance is cytokinin, which is produced primarily by the root system and known to be involved in flowering. We are investigating whether a significant correlation exists between root system cytokinin production and the ability to tolerate GFLV infection in a range of *X. index* resistant and susceptible rootstocks by comparing phytohormone levels with those from O39-16 and O43-43. Results from Freedom, St. George, 101-14 Mgt, 110R, and 10 *Vitis* x *Muscadinia* hybrids are compared.

### **The Endomycorrhizal Fungus *Glomus intraradices*: A Potential Biocontrol of *Cylindrocarpon* Black Foot Disease of Grapevine**

**Elsa Petit\*** and W. Douglas Gubler. Plant Pathology Department, University of California, Davis, CA 95616 [email: elpetit@ucdavis.edu]

*Cylindrocarpon* black foot disease occurs throughout the grapegrowing regions of the world where it is frequently a problem in vineyard establishment. The disease is a root and butt rot caused by fungi in the genus *Cylindrocarpon*. Below-ground symptoms include a reduction in total root biomass, low numbers of feeder roots, and sunken necrotic root lesions. Aboveground the vegetation is stunted and chlorotic, with leaf-scorch symptoms resembling water stress. Effective disease control programs have been elusive. Because the disease is more severe when plants are stressed and disease susceptibility decreases as vines age, the use of endomycorrhizae as biocontrol agents was explored. Our objective was to test whether the endomycorrhizal fungus *Glomus intraradices* is an effective biocontrol against black foot disease. After dormant *rupestris* cuttings were rooted in the presence of *G. intraradices*, vines were inoculated with *Cylindrocarpon* spp. Eight months after inoculation with the pathogen, disease severity was assessed. The pathogen decreased root dry weight by 30% and caused about 50% root rot ( $p = 0.05$ ). Root dry weights of plants co-inoculated with *G. intraradices* (mycorrhizal) and *Cylindrocarpon* spp. were significantly greater than root dry weights of nonmycorrhizal plants ( $p = 0.05$ ). Moreover, the number of root lesions were significantly reduced in *G. intraradices* inoculated plants ( $p = 0.05$ ). Preinoculation of plants with *G. intraradices* significantly decreased disease severity.

### **Allelic Relationship between Root-Knot Nematode Resistance Genes in a *Vitis mustangensis* Hybrid Population**

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Root-knot nematodes, *Meloidogyne* spp., are a major pest of grapes worldwide. Populations of *Meloidogyne* have been isolated that are capable of feeding and reproducing in the presence of the *N* allele, the main source of genetic resistance to root-knot nematodes in grape. *Vitis mustangensis* DVIT 1842, an accession in the National Plant Germplasm System, pro-

vides resistance to *N*-virulent nematode strains. Progeny testing determined that it is heterozygous for a single dominant allele conferring resistance to both *N*-virulent and *N*-avirulent nematode strains. We hypothesized that the new allele, which we designated *R*, is found at a distinct resistance locus and is not an allele of the *N* gene. The relationship of *R* to *N* was determined using progeny testing. The resistant accession (genotype nnRr) was crossed with the rootstocks Freedom and Harmony, homozygous for the *N* allele (NNrr). Progeny from the Freedom/Harmony x *V. mustangensis* DVIT 1842 crosses were screened with *N*-virulent nematodes, and resistant seedlings (genotype NnRr) were selected. These seedlings were treated with plant growth regulators chlormequat and N-benzyl-9-(2-tetrahydropyranyl) adenine (a cytokinin) to induce precocious flowering. Pollen was collected from flowering resistant seedlings and used to make test crosses with a nematode-susceptible female, the rootstock 161-49C (nnrr). Test cross progeny (NnRr x nnrr) were screened with *N*-avirulent nematodes and segregated 3:1 for resistance, indicating that *N* and *R* are nonallelic and represent two distinct resistance loci. This knowledge will facilitate the use of *V. mustangensis* DVIT 1842 in breeding to produce new rootstock cultivars resistant to *N*-virulent nematodes.

### **Creative Cover-Cropping Strategies for Willamette Valley Vineyards**

**R. Paul Schreiner\*** and **Rebecca M. Sweet**. USDA-Agricultural Research Service Horticulture Crop Science, 3420 NW Orchard Ave., Corvallis, OR 97330 [email: schreiner@science.oregonstate.edu]

Little is known about appropriate cover crops for use in cool-climate Oregon vineyards. It is estimated that 50 to 75% of Oregon vineyards are now tilling at least alternate vineyard alleys in order to (1) reduce water stress, (2) increase vineyard canopy temperatures, and (3) increase nutrient availability. Because many vineyards are situated on steep hillsides, frequent tillage will likely result in increased soil erosion, decreased soil quality, and potential pollution of watersheds. The focus of this research was to identify various cover-crop mixtures that will minimize drawbacks and maximize benefits of using cover crops in vineyard alleys as an alternative to tillage. In September 2003, seven cover-crop treatments were applied in a randomized block design at two commercial vineyards in the northern Willamette Valley. Treatments were clean cultivated, resident vegetation, subclovers, perennial grasses, cereals, native grasses, and native forbs plus grasses. Volumetric soil water content was monitored throughout the growing season and revealed decreasing trends in all treatments. Prune weights were low at both sites in the cereal treatment. Fine root density was higher in the vine row than in the alley at both sites at harvest but at only one site at bloom. Arbuscule frequency was higher in the vine row than in the alley at both sites at bloom and at one site at harvest. No differences were observed between treatments in soil water content or vine water stress or in fruit yield or juice quality.

## Viticulture Oral Session, Thursday

**Vine Water Relations of Field-Grown *Vitis vinifera* L. in Response to High-Frequency Drip Irrigation**

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A study was conducted on *Vitis vinifera* L. (cv. Thompson Seedless) to evaluate various measurements of vine water status under high-frequency drip irrigation. Water use at 100% of vine evapotranspiration was determined with a weighing lysimeter. Vines in the surrounding vineyard were irrigated at 0, 0.2, 0.6, 1.0, or 1.4 times the amount of water used by the lysimeter vines. Soil water content ( $\theta_v$ ) was measured in the 0.2, 0.6, 1.0, and 1.4 irrigation treatments. Predawn ( $\Psi_{PD}$ ), midday leaf ( $\Psi_l$ ), and midday stem ( $\Psi_{stem}$ ) water potentials were measured at the end of the 1991 and 1992 growing seasons and almost monthly during 1993. Soil water content in 1993 remained constant throughout the growing season for the 1.0 irrigation treatment; it increased in the 1.4 treatment; and it decreased in the 0.2 and 0.6 treatments. Both  $\Psi_l$  and  $\Psi_{stem}$  measurements detected differences among irrigation treatments to a greater extent than did  $\Psi_{PD}$  until very late in the 1993 growing season. There was a linear relationship between  $\Psi_l$  and  $\Psi_{stem}$ . All three measurements of water potential were related to soil water content; however, the relationship between SWC and  $\Psi_{PD}$  had the lowest  $r^2$  value, 0.52 compared to 0.90 and 0.94 for  $\Psi_l$  and  $\Psi_{stem}$ , respectively. A reduction in yield and pruning weights from 40 to 60% was measured when  $\Psi_{PD}$  decreased from -0.05 to -0.15 MPa. The results indicated that  $\Psi_{PD}$  would not be useful in accurately determining vine water status or its effects on productivity under high-frequency deficit irrigation.

**Merlot Vine Water Status Impacts Yield, Berry, and Wine Quality**

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The objective of this research was to determine how severity and phenological timing of vine water stress impacts yield components, berry, and wine composition. Vines in a one-acre trial established in southern Idaho within a commercial, 30-acre block of own-rooted, five-year-old Merlot were irrigated weekly from fruit set through harvest at percentages of estimated evapotranspiration (ET) needs during the 2002, 2003, and 2004 growing seasons. Levels of vine water stress were classified as low (100% ET), moderate (70% ET), or severe (35% ET) and were measured as midday leaf water potential. Yields of vines moderately or severely stressed were reduced 17% and 40%, respectively, with 11% and 30% reductions, respectively, in berry size. Severe water stress was associated with an increase in number of berries per cluster, a decrease in number of clusters per vine, reduced annual trunk growth, lower titratable acidity, and malic acid concentration, increased incidence of sunscald, and higher intensity wine. Severely stressed preveraison vines altered to a moderate level of stress at veraison through harvest had malic acid concentrations 10% higher and berry size 14% larger than vines severely stressed through harvest, yet berry size remained smaller

than low- or moderate-stressed vines with malic acid concentration and wine intensity similar to moderate-stressed vines. Results suggest a narrow margin between beneficial and potentially deleterious levels of vine water stress and show that stress severity altered at veraison attenuates the impact of severe preveraison stress.

**Leaf, Stem, and Predawn Water Potential for the Determination of Water Stress in Grapevine**

**Ken Shackel\***, Christine Barbe, and Xavier Chone. Department of Plant Sciences/Pomology, University of California, Davis, CA 95616 [email: kashackel@ucdavis.edu]

For the pressure chamber technique, a number of alternative sampling methods have been proposed for use as plant-based measures of water stress in grapevine. Predawn water potential (DWP) and midday stem and leaf water potential (SWP, LWP) are generally well correlated, as may be expected based on the current understanding of the soil-plant-atmosphere continuum. In grapevine and other woody perennial crop plants however, these alternative methods have not exhibited the same sensitivity to differential irrigation treatments or to soil conditions that are known to be associated with differences in water stress. Where differences have been exhibited among these methods, SWP has been the most sensitive. The progressive decline in depth of a water table in a commercial Pinot noir vineyard in Napa Valley was clearly reflected in a progressive decline in SWP from late June to late July, whereas over this same period of time, no change was exhibited in DWP. SWP exhibited statistically significant differences between irrigated and nonirrigated Pinot noir vines more than one month earlier in the season, and more consistently during the season, than LWP. Under field conditions, vine-to-vine differences in SWP have also been well correlated to vine-to-vine differences in growth and stomatal conductance. We propose that SWP is a suitable standard method for the determination of water stress in grapevine, as has been found in a number of other woody perennial crop plants.

**Worldwide Use of Stem Water Potential to Manage Vine Water Deficit with Irrigation in Estate Vineyards**

**Christine Barbe**, Cornelis Van Leeuwen, Ken Shackel,\* and **Xavier Choné**. Department of Plant Sciences/Pomology, University of California, Davis, CA 95616 [email: kashackel@ucdavis.edu]

Midday stem water potential (SWP) has been used to manage vine water deficit through irrigation and to guide vineyard management decisions related to water availability (rootstock, planting density, canopy management) when replanting both irrigated and nonirrigated vineyards. SWP data covering a range of locations (Italy, Morocco, Greece, Spain, South America, California, Burgundy, Bordeaux, Champagne, Rhone) and various grafted or own-rooted *Vitis vinifera* cultivars (Cabernet, Merlot, Syrah, Pinot noir, Sangiovese, Chardonnay, Sauvignon, Carmenere, Tempranillo, Agiorgitiko) were surveyed. The influence of soil water holding capacity (SWHC), as estimated from soil texture, structure, root-zone depth, and presence of a temporary water table, was also examined. The overall range of SWP observed in *V. vinifera* was from -0.3 to -1.8 MPa for a wide range of climates, variations



in SWHC (<100 mm to >400 mm), and variations in leaf area (6000 to 20000 m<sup>2</sup> of leaf area per hectare). We suggest that the French terroir theory may be principally founded on vine water deficit effects of the soil-plant-atmosphere continuum. Based on this hypothesis, irrigation experiments were conducted in two Napa Valley locations, with irrigation management guided by either SWP and SWHC or ETc and leaf water potential. Differences in irrigation management decisions were striking, particularly for vineyards with high SWHC. Delaying irrigation was indicated using SWP and SWHC, while irrigation was recommended using ETc and leaf water potential. For the production of estate quality fruit and wine, irrigation must be managed to maintain the vine canopy at a moderate level of water deficit through harvest.

### Structural Integrity of Xylem in the Postveraison Grape Berry

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Both phloem and xylem contribute to berry water requirements at preveraison. However the phloem predominates in postveraison berries. Studies based on anatomical and dye-infusion techniques seemed to indicate that the xylem conduits within the berry lose integrity and become dysfunctional at veraison. A physical break or gap was reported in the xylem of postveraison berries in the earlier studies. However, new experiments have shown that apoplastic dye is able to move throughout the peripheral xylem strands in postveraison berries. The existence of xylem gaps was reevaluated using maceration and plastic embedding techniques. The secondary wall thickening (gyres) of individual tracheids was observed in berries at preveraison, veraison, and postveraison stage. No xylem breaks or discontinuity could be found in the peripheral xylem strands dissected from different locations in the berry. The gyres are uniformly spaced within a tracheid. However, the distance between them increases at veraison (18 to 25%) to reach 42% at postveraison. In addition to the dye experiments, these results proved that the xylem does not become physically disrupted during berry development but is able to stretch in response to the berry enlargement. Consequently, some other factor must either decrease the driving force or increase the xylem resistance to water flow in the postveraison berries.

### Effects of Regulated Deficit Irrigation on California North Coast Cabernet Sauvignon

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In California's North Coast, achieving moderate, consistent vine water deficits is complicated by annual variations in early season rainfall. A four-year irrigation strategy study (1997 to 2000) was conducted in a 1.4 acre Cabernet Sauvignon vineyard, clone 8 on 5C rootstock, at the University of California Hopland Research and Extension Center. A regulated deficit irrigation approach was investigated using leaf water potential levels of -1.2 and -1.4 MPa to trigger the start

of irrigation followed by deficit irrigation at 35 or 60% of full potential water use. Additional treatments were 100% full potential water use and a variable deficit irrigation strategy. Leaf water potential in the deficit irrigation treatments dropped below that of the full water treatment before veraison, and leaf water potentials declined more in the treatments receiving less water. Shoot length and pruning weights declined, and light in the fruit zone increased with reduced applied water. Yield reductions in the deficit treatments relative to the full water treatment were 12 to 18% the first year and stabilized at 21 to 37% by the third year of the study. Delays in soluble solids accumulation were observed in the treatments experiencing the most severe water deficits. Titratable acidity and malic acid concentrations were higher in treatments receiving more irrigation water, while irrigation treatment effects on juice pH and potassium concentration varied from year to year. Leaf water potential used as a trigger to initiate irrigation followed by application volumes that were portions of full potential water use gave consistent effects on yield, juice composition, and vegetative growth.

### Enology Oral Session, Thursday

#### Detection of 2-Aminoacetophenone Off-Flavor in Wine by Solid-Phase Microextraction and SIM GC-MS

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2-Aminoacetophenone (2-AAP) was first reported by a German research group in untypical aging (UTA) off-flavor white wine, which was described as furniture polish, wet wool, mothball, fusel alcohol, and acacia blossom. Because of its low threshold (0.5 to 1.5 µg/L), many researchers suspect that it is the major compound responsible for UTA off-flavor. Given the increase of UTA off-flavor in American white wines, understanding the behavior of this compound in wine has become more important. In this work, a sensitive solid-phase microextraction (SPME) coupled with selective mass ion detection technique was developed to determine 2-AAP in wine. A 20-mL wine sample was placed into a 40-mL vial and saturated with sodium chloride. The saturated sample was preequilibrated for 15 min and extracted using DVB/CAR/PDMS fiber for 30 min at 50°C with stirring. A DB-5 column was used for separation and the selective mass ions (*m/z* 135 and *m/z* 120) used for quantification. The method can detect 2-AAP in wine samples at 0.5 µg/L, which is at the sensory threshold. Different wine samples were investigated by this method.

#### Rapid Immunoassays for Stable *Botrytis* Antigens in Pre- and Postsymptomatic Grape Berries, Grape Juice, and Wines

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We have developed a rapid (6 min), user-friendly immuno-chromatographic assay or lateral flow device for *Botrytis*. This device employs a *Botrytis*-specific monoclonal antibody, BC-

12.CA4, that recognizes a water-soluble antigen. The antigen is produced constitutively and is present in the walls and extracellular matrix of *Botrytis*-hyphae but not on the surface of the spores. It is highly stable and is not degraded or metabolized during fermentation. The *Botrytis*-lateral flow device was designed for use on test stands at wineries at harvest time and in the field. The device is semiquantitative; the time of appearance of the test band is related to the concentration of *Botrytis* antigens in the sample. In tests with juice or wines (including champagnes) made from visibly infected material, the test band appears rapidly within 1 to 3 min; with extracts from presymptomatic material the test band appears more slowly, generally within 15 to 25 min. The intensity of the test band, at any one time, is also related to the level of *Botrytis* antigens in the sample. The intensity of the test band can be measured with a reflectometer. We have been using a custom-made reflectometer that automatically scans and determines the intensity of the test band at 6 min. Results from tests with juice samples obtained in the 2003 and 2004 harvests correlate well with hand-sort methods of determining the level of rot in grapes.

### **Lysozyme Interaction with Anthocyanin and Pigmented Polymer Fractions from Pinot noir Wine**

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Egg white lysozyme is used in wine to prevent growth of undesirable lactic acid bacteria. Lysozyme can precipitate wine components resulting in anthocyanin/tannin loss and reduction in lysozyme activity. Understanding lysozyme interactions in wine will optimize its use as a wine antimicrobial. We evaluated the reaction between various wine fractions and lysozyme by analyzing color, enzyme activity, turbidity, and rates of haze formation. Variables were temperature and reactant concentration ratios. Monomeric anthocyanin from Pinot noir wine was solubilized in 0.5% w/v tartaric acid to 500 mg/L and was adjusted to pH 3.5. Lysozyme (50, 150, 300, and 500 mg/L) was added and held for 24 hr with absorbance read at 280, 420, 520, and 620 nm. Lysozyme addition to monomeric anthocyanin did not elicit any change in absorbance at any wavelength except at 280 nm, which is explained by lysozyme protein absorption. Sediment or haze was not observed. A pigmented polymer was obtained from three-year-old Pinot noir wine. Addition of lysozyme to pigmented polymer resulted in immediate formation of a colloidal haze with particles in excess of 25  $\mu\text{m}$  as seen with dark field microscopy. Rates of haze formation were determined by monitoring absorbance (420, 520, 620 nm) at 15, 20, 25, and 30°C over time. Maximum haze formation was >90% complete within 30 min for all variables. Concurrent with haze formation was loss of color and of lysozyme activity.

### **Viticulture Oral Session, Friday**

#### **Evaluation of Abscisic Acid for Budburst Delay of Grapevine**

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Spring frost damage is a significant production risk in many grapegrowing regions. Damage from a spring frost event could often have been avoided if grape buds had not yet begun to grow. This research evaluated an experimental formulation of abscisic acid (ABA) for grapevine budburst delay. Preliminary trials were conducted in 2004 on dormant cuttings, potted vines, and field-grown vines to evaluate rates and timing of ABA applications. ABA solution was sprayed on single bud, dormant cuttings of Sangiovese. Treatments consisted of four rates of ABA (0, 10, 100, or 1000 mg/L) and five application dates. There was no significant effect of ABA rate on budburst of dormant cuttings. However, timing of ABA application influenced budburst, with the greatest delay resulting from later treatments. ABA solution applied as a soil drench to container-grown dormant vines of Cabernet Sauvignon and Sangiovese at rates of 0, 10, 100, or 1000 mg/L significantly delayed budburst. The highest rate reduced budburst for as long as three weeks, but also reduced the percentage of buds that grew. Field-grown vines of Sangiovese were treated on four dates with three rates of ABA (0, 100, 1000 mg/L) applied as a spray to dormant buds. Vines treated with ABA had a significant reduction in percent budburst at the earlier evaluation dates and both rates performed similarly.

#### **Grapevine Leaf and Fruit Tissue Responses to Fosetyl-Al and UV Radiation**

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Fosetyl-Al (aluminum tris (*o*-ethyl phosphonate)) has been implicated in grapevine phytoalexin or induced resistance responses in a number of studies. Here, grapevine tissues were subjected to fosetyl-Al and UV treatment to investigate the phytoalexin response, detected by *trans*-resveratrol production. Detached leaves treated with different concentrations of fosetyl-Al and then dosed with UV radiation showed a positive *trans*-resveratrol response, with *trans*-resveratrol concentration peaking at two days. However, without the UV-elicitation event, fosetyl-Al had no effect on *trans*-resveratrol. Leaves left on the vine, unlike detached ones, showed a direct effect of fosetyl-Al on *trans*-resveratrol production, with its concentration peaking at four days. Immature fruit on the vine showed a different response to fosetyl-Al treatment, with *trans*-resveratrol concentration peaking at one day and falling to zero soon after. The effects of fosetyl-Al and UV radiation on *trans*-resveratrol did not appear additive. Our findings indicate that experiments on at least vine leaves using fosetyl-Al should take place in situ, as responses in terms of *trans*-resveratrol differed greatly compared to leaves in vitro.

## Evaluation of Mechanical Pruning on Cynthiana Grape and Wine Composition

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Vineyard mechanization has the potential to reduce vineyard production costs while maintaining yield, fruit, and wine quality and viticultural soundness. This experiment was established on Cynthiana (*Vitis aestivalis* Michx.) to compare two mechanical pruning methods to hand pruning. Three pruning treatments were used: HAND, hand prune to 50 + 10 with an 80-bud maximum; MPBC, machine pruning-box cut, leaving 70 to 80 buds; and MPHT, machine pruning-box cut, hand touch-up leaving the best 80 buds. Plant tissue, berry, and cluster samples were collected and laboratory analyses run for each field replicate. Wine was made from each treatment. HAND, MPBC, and MPHT treatments had similar values for plant tissue minerals, yield, fruit sugars, organic acids, color, minerals, and total phenolics within year for 2002, 2003, and 2004. MPBC treatments had smaller and fewer clusters per node than other treatments. Wine composition values for 2002 and 2003 wines showed little or no difference among treatments. Sensory analysis of the 2002 wines showed no differences among treatments. Three years of data indicated that MPHT and MPBC pruning produced yield and fruit composition equivalent to HAND pruning. Preliminary results for wine indicate little or no difference among pruning treatments. The use of mechanical pruning on Cynthiana grapes appears to produce fruit and wine of similar composition to hand pruning.

## Wine Sensory Analysis Shows Different Responses to Pruning, Thinning, and Irrigation Regulated Yield

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Yield and irrigation are two of the most commonly regulated components of winegrape production, but chemical analysis of the fruit produces limited information about the wine sensory attributes that result from that regulation. The dependence of wine sensory properties on yield and vine water status was tested using Cabernet Sauvignon vines in the Napa Valley. When yield was varied by pruning or cluster thinning, yield varied from 4.3 to 22.2 t/ha. When irrigation was varied, grape yields varied from 15.0 to 21.7 t/ha. Analysis of variance and principal component analysis showed that the wines made from vines pruned to low node numbers (hence low yield) were higher in vegetal aroma and flavor, bell pepper aroma, bitterness, and astringency than high-yield wines. Conversely, the wines made from vines pruned to high node numbers were higher in red/black berry aroma, jam aroma, fresh fruit aroma, and fruity flavor than low-yield wines. There were few sensory differences detected in wines made from the various cluster-thinning treatments. Wines made from the minimal irrigation treatment were significantly higher in red/blackberry aroma, jam/cooked berry aroma, dried fruit/raisin aroma, and fruit by mouth than the wines from the two higher irrigated treatments. Thus, when manipulated by irrigation, yield had

an inverse relation with wine flavor; the low-yielding vines produced wines with the most fruity and least veggie sensory notes. Therefore, the viticulture practices used to control yield in a vineyard may be more important than the yield values per se in determining the sensory properties of the resulting wines.

## Innovative Forms of Diversity in California Vineyards: Integration of Biodiversity and Crop Diversification in Winegrape Production Systems

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Although viticulture in California is usually considered a monoculture, increasing numbers of winegrape vineyards are incorporating biodiversity enhancement practices and crop diversification schemes. This analysis provides a summary of several vineyard case studies that feature innovative forms of diversity, including the growers' motivations for using these innovations and the results in terms of yields, economics, and indicators of ecosystem health. Results show that these vineyards establish conditions that have multiple benefits to the vineyard system and help create balanced winegrape production. The cases illustrate how the use of a "nonmonocultural" approach can be successful. While the overall economic benefits and costs are difficult to quantify, these innovative vineyard operations reveal positive outcomes in terms of yields and ecosystem functions.

## Mechanical Crop Adjustment: A Successful Research and Extension Effort in the Lake Erie Region

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Research on the impacts of crop load on Concord grape quality has been conducted for several decades at Cornell University's Vineyard Laboratory in Fredonia. Work in recent years has focused on adapting the results of this research into tools that growers could use to adjust crop levels in their vineyards during the growing season. In 2003, many Concord vineyards in the Lake Erie region were significantly overcropped because of above-average berry set. This fact, combined with a cool and wet growing season, caused great concern among growers about their ability to market their crop and among processors about the quality of the crop they would receive. Lake Erie Regional Grape Program research and extension staff worked with area processors to introduce and demonstrate mechanical crop estimation and thinning techniques to growers for use in their own vineyards. A survey was developed after the season was finished to assess the level of adoption of the practices, what specific techniques were used, and growers' intentions for their future use. The survey indicated that approximately 25 to 30% of the acreage in the region was thinned and that 35 to 40% of growers adjusted the crop on some portion of their acreage. The area's largest grape processor estimated that the development and dissemination of this information resulted in the harvest of \$3.6 million worth of Concord grapes that otherwise would not have been marketable.



### Method for Drying Raisins on the Vine That Alternates Fruiting Canes and Renewal Shoots within the Row and Uses a Conventional Trellis

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A method to dry raisins on the vine was developed that allows the use of a traditional raisin trellis, referred to as WRAB DOV. WRAB DOV separates fruiting canes and renewal shoots by vine section down the row, with a vine section the space between two adjacent vines. The yield of Thompson Seedless by WRAB DOV compared with traditional tray drying was evaluated over five seasons. WRAB DOV yield was similar to tray drying, but raisin grade was consistently higher with dried on vine at the same fruit maturity. WRAB DOV raisins were easily harvested by a canopy shaker winegrape harvester. Shoot thinning the head of Thompson Seedless prebloom increased cane fruitfulness in years when climate was not optimum for initiation of flower cluster primordia. Vertical trellises with single and double wires were compared with T trellises having crossarm widths of 0.45, 0.9, and 1.2 m (2, 3, and 4 wires, respectively). Raisin yield was positively affected by increasing the width of the cross arm, with no difference in berry weight or fruit maturity. Yield increase was 14% per 30 cm of crossarm width in 2003 and 21% in 2004, which was attributed to higher numbers of shoots and flowers emerging from fruit canes in the spring and on increased canopy size.

#### General Enology Poster Session

### Influence on Wine Flavor and Fermentation of Non-*Saccharomyces* Yeasts: Results of 24 Field Trials at 15 Wineries

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Non-*Saccharomyces* yeasts are thought to affect the sensory profile of wine significantly, although scientific data that explains this impact is scarce. In 2003 to 2004, 24 full-scale trials were set up, primarily in California, to investigate starter cultures containing selected non-*Saccharomyces* yeasts and their effect on wine fermentation and flavor. The trials were blind with coded cultures. In addition to the pure *Saccharomyces cerevisiae* control culture, the wineries received mixed cultures containing *Torulaspora delbrueckii* and/or *Kluyveromyces thermotolerans*. Fermentation data and wine samples for sensory evaluation were collected. Partial least square regression (PLS) of the fermentation data grouped the starter cultures according to their non-*Saccharomyces* content. It also showed that the non-*Saccharomyces* starters had little technical impact on the fermentation; the effect of varietal and winery practices was greater. According to PLS, non-*Saccharomyces* cultures had considerable but different effects on Chardonnay and Riesling. Nine of the trial wine sets underwent sensory testing in July 2004. Analysis was done by triangle testing. The predominant varietal for sensory tested

wines was Chardonnay, and the *K. thermotolerans*/*S. cerevisiae* mix showed the greatest effect in this varietal. A sensory evaluation of a German Riesling from a separate trial with the non-*Saccharomyces* cultures associated non-*Saccharomyces* with enhancement in fruitiness and body and gave lower notes on acidity and bitterness.

### Production of Volatile Phenols by Lactic Acid Bacteria

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Some wine microorganisms can produce volatile phenols (4-vinylphenol and 4-ethylphenol) from wine phenolic (*p*-coumaric and ferulic) acids. Volatile phenols have characteristic aromas that, above a certain concentration threshold, have a negative effect on the overall aroma of a wine but at low concentrations have been cited as contributing positively to aroma complexity. Previous works showed that some strains of lactic acid bacteria (LAB) can produce low concentrations of volatile phenols in near-wine conditions. In this work, 35 strains of LAB (19 species) were screened for their ability to produce volatile phenols from the corresponding phenolic acids. Cells were cultivated in liquid media supplemented with *p*-coumaric or ferulic acid at 500 mg L<sup>-1</sup>. The concentration of volatile phenols in the growth media was analyzed by GC-FID. Results indicated that 13 strains (37%) were able to produce volatile phenols from *p*-coumaric acid, although only three (9%) produced 4-ethylphenol as the final product. Seven strains (20%) were able to convert ferulic acid to 4-vinylguaicol but none produced 4-ethylguaicol. Seven (of eight) strains of *Pediococcus* and six (of 25) strains of *Lactobacillus* produced volatile phenols from phenolic acids in sensorially significant amounts. The two *Oenococcus oeni* strains studied did not produce volatile phenols. Strains which were found to produce volatile phenols were used in subsequent studies at lower phenolic acid concentrations. Experiments with added 5.0 mg L<sup>-1</sup> of *p*-coumaric acid showed that some strains can still produce relatively high (up to 800 µg L<sup>-1</sup>) concentrations of 4-ethylphenol.

### Development of a High-Throughput SO<sub>2</sub>-Tolerant Assay to Measure Laccase Levels in Juice and Wine

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Laccase is a fungal enzyme primarily associated with rot on grapes caused by the grape pathogen *Botrytis cinerea*. Laccase can negatively impact wine quality by causing premature browning of bottled white wine and color degradation in red wine. The current commercially available laccase assay does not function reliably in the presence of a moderate level of SO<sub>2</sub> (20 to 50 mg/L). We have developed a method for measuring laccase activity in finished wine and/or test stand juice samples containing a moderate level of SO<sub>2</sub>. The assay uses a glycine-HCl buffer system (50 mM, pH 3.5) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS). Addition

of 0.002% ascorbic acid to the buffer inhibits nonspecific oxidase activity in juice samples. A high-throughput method was developed using the assay in a 96-well format. Wine/juice samples were filtered through a quartz sand:polyvinylpyrrolidone (3:2) matrix using a 96-well vacuum manifold. Assay buffer (70  $\mu$ L) was added to each well of a 96-well plate, followed by the addition of 50  $\mu$ L of processed juice/wine sample. 30  $\mu$ L of ABTS (4.65 mM) was added to each well and the plate was loaded into a Spectronics SpectraMax Plus UV/Vis plate reader. A kinetic assay program automatically calculated laccase activity. One person can assay approximately 600 samples in an 8-hr shift.

### Effect of Cryoextraction on Phenol Fractions of Musts Derived from White Grape Varieties

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Cryoextraction is a practice of subjecting grape to low temperature (<0°C) before pressing to improve quality traits and aromatic properties of white wine. As a result of the cryoextraction process, grape enzymes are provisionally blocked and only the premium juice is expressed because the not perfectly ripe grapes are still frozen. This technique was applied to traditional white grape varieties of the Verona area using liquid nitrogen as a cryogenic agent. Different freezing temperatures were compared. During the pressing procedure, there were marked differences in the main must components. Wide extraction of total phenols, catechin, and proanthocyanidins was observed, which was probably due to partial skin damaging as a consequence of low temperature treatment. The extent of this extraction depended on the treatment temperature and the grape variety. For this reason, the application of this technique requires particular attention to enological practice, in particular the use of antioxidants, such as sulfur dioxide, because of the risk of phenolic compound oxidation and browning of wines. The aim of our research was to evaluate the effect of grape freezing on polyphenol extraction and to optimize the technique of cryoextraction in relation to a protective antioxidant effect.

### Effect of Different Environmental Conditions on Berry Polyphenols during Postharvest Dehydration of Grapes

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In Valpolicella, a hilly area northwest of Verona, Italy, typical Recioto and Amarone wines are obtained following the dehydration (up to 35 to 40% of water loss) of harvested grapes that are placed in drawers and stored in specialized areas where withering occurs because of favorable meteorological conditions. Withering techniques of grapes are evolving with the use of technology aimed to control the process (through modulation of environmental parameters such as temperature, relative humidity, and ventilation), thus affecting the dehydration rate. Here we report the effect of different storage

conditions on the major phenolic compounds of grape. Clusters of cv. Corvina were harvested and immediately stored at three different ventilation regimes in order to induce three different rates of dehydration: the same water loss was reached in 70, 85, 90 days, respectively. The different rate of dehydration affected sugar and organic acid concentrations and induced significant changes in all major phenolic fractions analyzed. The level of these classes of compounds decreased during the first period of drying and increased thereafter, reaching higher levels in grapes dehydrated for longer times. This behavior may be attributable to an initial oxidation/polymerization followed by an increase of extractability probably due to changes in cell ultrastructure (loss of compartmentation and/or of cell wall integrity). These data indicate that the post-harvest dehydration rate, modulated by different ventilation conditions during storage, has a great impact on berry composition and/or extractability of the main polyphenol fractions, with potential effects on quality traits of wines.

### Nitrogen Requirement during Alcoholic Fermentation by Wine Yeast Strains

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The objective of this work was to evaluate the fermentability of a synthetic grape must according to its assimilable nitrogen content for 10 commercial wine yeasts. The growth and fermentation kinetics were determined as well as some characteristics of the fermented medium. The assimilable nitrogen concentration of the must varied from 120 to 290 mg N/L for an initial concentration of reducing sugars of 240 g/L. The amount of consumed sugars was correlated to the amount of consumed nitrogen for most of the strains, but in some cases an optimal concentration was noted. For the highest initial nitrogen concentrations, a notable proportion remained after completion of alcoholic fermentation and in some cases the fermentative rate was not improved. A nitrogen catabolic repression could be then assumed, leading to a decreased fermentation rate for higher concentrations. For each condition the nitrogen uptake could be calculated as mg N by g of consumed sugar, and thus it was shown that the richer in nitrogen the medium was, the higher its consumption was without increasing the final biomass concentration but increasing the acetic acid production. It is suggested that the nitrogen could be consumed for de novo protein synthesis by yeast cells. Results indicate that addition of ammonium salts in wine-making should be done cautiously to avoid undesirable consequences ( $H_2S$ , acetic acid, ethyl carbamate or higher alcohols inappropriate production) and not exceed yeast requirement.

### Douro Grape Characterization: Carotenoid Profile in Grapes Related to Aromatic Compounds in Wine

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The aim of this work was to characterize eight representative grape varieties of the Douro region using the carotenoid

profile as it relates to aromatic compounds in the respective wines. Some other analyses, such as the determination of sugar, probable alcohol, pH, and total acidity, were also performed in an attempt to understand in which ways the evaluated characteristics influenced by grape variety could contribute to wine aroma. Differences among cultivars were observed in the eight black grape varieties. Touriga Fêmea (TFê) clearly produced a higher concentration of carotenoids in all three years of the study. Tinta Amarela (TA) also showed higher carotenoid levels in 2001 and 2002. Although TFê and TA have produced higher concentrations of carotenoids, these cultivars have not necessarily produced the most aromatic wines. Touriga Nacional (TN), followed by TFê, was the wine variety with the highest values of total free terpenols (linalol,  $\alpha$ -terpineol, nerol, and geraniol), the presence of which is responsible for the floral aroma, and  $\beta$ -ionone, described as having a violet-like character. Sousão and Tinto Cão had the lowest concentrations of carotenoids. Conversely, these cultivars produced higher concentrations of vitispirane and TDN, two important norisoprenoids, the presence of which is related to eucalyptus/camphor and kerosene descriptors, respectively.

### Identifying Characteristic Volatiles of Frontenac Wine by Stir Bar Sorptive Extraction, GCO/FID, and GCO/MS

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Frontenac is a new, cold-hardy red grape hybrid of increasing economic importance in nontraditional wine-growing regions. As a new cultivar, little is known about the aroma and flavor compounds expressed in Frontenac wine. In this study, eight commercial and research wines were analyzed for volatile and phenolic compounds. For volatile characterization, stir bar sorptive extraction (SBSE) was performed. A 10-mm stir bar coated with polydimethylsiloxane (PDMS) was equilibrated in a 20-mL wine sample for 1.5 hr, then desorbed and analyzed by gas chromatography (flame ionization detector)-olfactometry (GCO/FID) and/or gas chromatography olfactometry/mass spectrometry (GCO/MS). Wine phenols were analyzed via spectrophotometric measurement, Folin-Ciocalteu, and the modified Adams-Harbertson method. Several GC peaks were found in the chromatograms of all wines examined; many of these also correlated with key aromas recorded by olfactometry. Ethyl octanoate, phenethyl acetate, and ethyl-9-decenoate were among the volatiles found to be potentially important to Frontenac aroma. Phenol analysis suggests that high total phenols and pigmented compounds are characteristic of this hybrid.

### Effect of *Glomerella* on the Physicochemical Composition of Cabernet Sauvignon Wine

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In the last five years *Glomerella cingulata* has been one of the most important diseases of the Serra Gaúcha vineyards, Brazil's most important viticultural region. With humidity and

rain, the pathogenic agent spreads rapidly on the surface of berries and can cause grape ripe rot on the entire cluster, which later may shrivel. This disease decreases vineyard yield and affects wine quality. For this reason, an experiment was carried out to determine the effect of different levels of the disease on the physicochemical composition of Cabernet Sauvignon wine. Six treatments—grapes with 0, 2.5, 5.0, 7.5, 10.0, and 20.0% weight basis, infected with *Glomerella*—and three replications were used. The experimental design was a randomized complete block. Wines were made in 20-L glass recipients. Variables related to classical analysis, minerals, and volatile compounds were analyzed. Polynomial regression analysis showed that *Glomerella* significantly increased contents of alcohol, pH, dry extract, ashes, alkalinity of ashes, hue, methanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol and some minerals (N, P, Ca, Mg, Zn, Rb). However, density, titratable acidity, tannins, A520, A620, color intensity, and anthocyanins decreased. These results show that wine color variables were the most affected by *Glomerella*. There was no significant effect on reducing sugars, alcohol/dry extract ratio, A420, total polyphenols, tartaric and malic acids, ethyl acetate, and acetaldehyde.

### Influence of Maceration Methods on Total Phenolics, Color, and Lees Characteristics during Fermentation of Red Wine from Frozen Muscadine Grapes

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The purpose of this research was to study the feasibility of winemaking using frozen Muscadine grapes (*Vitis rotundifolia*). Spectrophotometric determination of total phenolics and color changes were recorded at different time intervals to evaluate the quality of wines during the fermentation of grapes subjected to cold (10 to 15°C for 60 and 120 hr), conventional, and carbonic maceration methods. The results indicated that wines produced from cold (60 and 120 hr) and conventional methods had significantly higher total phenolics and color index than wines produced from carbonic maceration methods. In addition, there were significantly fewer lees in wines produced from 60 and 120 hr cold maceration methods than in wines produced from conventional and carbonic maceration methods. Lees appearance evaluation indicated that the cold maceration method resulted in higher density lees that were easier to remove during racking than lees from carbonic and conventional maceration methods. However, different maceration methods did not affect the pH and total titratable acidity during wine fermentation.

### Ergothioneine as an Antioxidant and Potential Marker in Wine

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The presence of L-ergothioneine in wine may be a result of grapevine activity or biochemical processes that occur in juice and wine during fermentation. Samples were collected from three separate wines (two Pinot noir and one Pinot blanc)



and analyzed for L-ergothioneine using a direct external fluorescent labeling method after high-speed clarification of the samples. Samples were then separated by HPLC and quantified using a fluorescence detector and an internal standard. Standards were made from synthetic L-ergothioneine. Analytical results for the wines indicated L-ergothioneine concentrations ranging from 2.0 to 4.0 mg/L. Since L-ergothioneine is produced in the soil by fungi and certain mycobacteria, these results suggest potential translocation from the soil by *Vitis vinifera*. Biosynthesis of L-ergothioneine by fungi may also suggest potential involvement of *Saccharomyces* during fermentation. Wine could be a potentially significant dietary source of L-ergothioneine since conservative estimates indicate that the average human may consume approximately 1.0 to 2.0 mg of L-ergothioneine per day through a normal diet. Studies are being conducted to measure the presence of L-ergothioneine in grapevine material and determine whether L-ergothioneine in wine originates from the grapevine or during the winemaking process. The potential for L-ergothioneine to be used as a marker for grapevine stress is also being investigated.

### Improvement of the Fermenting Capacity of Active Dry Yeast by Solubilized Sterols

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During rehydration in aqueous media, active dry yeasts (ADY) could be supplemented with inactive yeasts, yeast derivatives, or other optional complementary nutrients in order to finally improve their fermentation capacity. We found that yeast sterols solubilized in situ during ADY rehydration were particularly efficient for the stimulation of the fermenting capacity of ADY. Spontaneous solubilization of sterols during rehydration occurred by formation of micelles between membrane phospholipids, specific cell wall polysaccharides and sterols, both compounds provided by inactive dry yeasts. These micelles contained a specific repartition of the initial sterols from the inactive yeasts. Above a concentration of 100 mg L<sup>-1</sup> in the rehydration medium, they acted as emulsifiers. Their critical micelle concentration was found to be in the order of 4.0 g L<sup>-1</sup>. During rehydration, purified micelles, at a concentration near the critical micelle concentration, were able to interact rapidly with yeast cell membranes by modifying the yeast plasma membrane order (monitored by steady-state fluorescence anisotropy of TMA-DPH probe) and by increasing the sterol contents of ADY. Such an enrichment of ADY by very low concentration of solubilized sterols was very efficient for the completion of fermentations, when musts are limited in available phytosterols or when microoxygenation is not desirable during fermentation.

### Rehydration Parameters on Active Dry Wine Yeast Lag Phase and Fermentation Activity

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Active dry wine yeast (ADWY) should be rehydrated before inoculation into juice and must. Currently, there are standard rehydration protocols, but these protocols were designed over 20 years ago when a small number of selected ADWY strains were available. Recent studies point out differences in wine yeast rehydration behaviors. In this work we studied the effect of the main rehydration parameters (temperature, water hardness, sugar concentration, rehydration time, and agitation) on the recovering fermentative activity of the yeasts. Three ADWY strains, showing differences regarding their rehydration behavior and/or their lag phases, were compared. Several rehydration parameters had a significant effect on the yeast's lag phase, confirming the importance of following ADWY manufacturer guidelines for proper rehydration. We also looked for early indicators of ADWY fermentation activity by studying three parameters during the rehydration phase: pH, released CO<sub>2</sub>, and trehalose utilization. We compared these parameters to two other parameters measured at the beginning of fermentation: the dissolved CO<sub>2</sub> concentration and lag phase duration. Some relationships were demonstrated, indicating the potential value of monitoring ADWY rehydration to help predict fermentation activity.

### Role of Various Fungal Species on French Oak Wood Volatile Compound Formation

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European barrel makers and alcoholic beverage producers prefer to use naturally aged oak staves because the staves can provide desirable aroma components during the barrel aging of alcoholic products. Since oak stave species are structurally and compositionally different, this can influence the volatiles in alcoholic beverages. Recent research using the electronic tongue approach has shown that the most important changes occur during the first year to 18 months. Some researchers have also investigated the evolution of ellagitannins during stave aging, but little work has focused on the resulting volatiles. During the aging of oak staves in the open environment, numerous fungal species can actively cover stave surfaces, thereby influencing the final volatiles. In this study, two different oak species (*Quercus pendunculata* and *Q. sessiliflora*) and three different fungal species (*Cephaloscypha fragrans*, *Aurebasidium pullulans*, *Penicillium roqueforti*) together with nonfungal controls were aged for one year in a model system. The resulting volatiles were identified and quantitated. A total of 24 compounds including lactones, acids, aldehydes, and phenols were identified. They ranged from 46.8 µg/g of dry wood to 134 µg/g, based on both wood and fungal species. The most active fungal species was *C. fragrans*, while the least active was *A. pullulans*. These data clearly demonstrate that the presence of fungi during the aging of staves can significantly influence resulting volatiles. Also, a minimum period of one year of drying oak staves is indispensable.

### Volatile Sulfur Compounds in Oregon Wines Determined by Solid-Phase Microextraction-Pulsed Flame Photometric Detection

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Volatile sulfur compounds can be positive attributes or may cause reduced off-flavor in wine. In order to understand sulfur compound formation in wines, a quantitative and sensitive method was developed using solid-phase microextraction (SPME) and GC-pulsed flame photometric detection (GC-PFPD). Eleven sulfur compounds could be quantified by this method: hydrogen sulfide, methanethiol, ethanethiol, dimethyl sulfide, diethyl sulfide, methyl thioacetate, dimethyl disulfide, ethyl thioacetate, diethyl disulfide, dimethyl trisulfide, and methionol. Most can be detected at ppb and some can be detected at ppt levels. In addition, 43 wine samples from eight major Oregon wineries were obtained and the sulfur concentrations in these wines were analyzed. Evaluated by sensory panel, four of them have sulfurous off-flavor. For normal wines, the instrumental results showed that the mean of hydrogen sulfide was 6.43 ppb (SE = 0.70), the mean of methanethiol was 2.69 ppb (SE = 0.43), and there was no ethanethiol. For these wines with sulfurous off-flavor, hydrogen sulfide (mean = 61.74 ppb, SE = 13.04) and methanethiol (mean = 5.25 ppb, SE = 0.68) were much higher concentration, and ethanethiol was also detected, which means these three high volatile sulfur compounds are major elements for wine sulfur off-flavor.

### Analysis of Head Space Gas of Oxidized White Wine

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The changes in the flavor profiles of white wine undergoing deterioration with and without oxygen were investigated by gas chromatography-olfactometry of headspace gas. The study showed that several odorants were generated during wine deterioration with and without oxygen and that several odorants decreased during the deterioration. The identification of the odorants was achieved by purge and trap multi-dimensional gas chromatography mass spectrometry. The odorants that increased during deterioration were identified as isobutanol, 2-methylbutanol, 3-methylbutanol, methionol, and phenylacetaldehyde. The odorant that decreased was linalool. White wine was stored with and without oxygen at 20, 30, and 40°C, and the time course of these aldehydes, 1,1,6-trimethyl-1,2-dihydro-naphtharene, free sulfite, bound sulfite, OD420, and sensory score were determined for four weeks. The results were analyzed by principal component analysis. Oxidized white wines were clearly distinguished by this analysis from forced aged white wines without oxygen.

### Flavor Enhancement: Mixed Starter Cultures of *Saccharomyces cerevisiae*, *Kluyveromyces thermotolerans*, and *Torulaspora delbrueckii*

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Spontaneous alcoholic fermentation of grape juice provides a range of different flavor impacts in wine as a consequence of the metabolism of indigenous yeast species present. The wine is often considered more complex, but inconsistent in quality, because of the interspecies competition between different yeasts occurring from year to year. Introducing *Saccharomyces cerevisiae* as starter culture in winemaking gave less problematic alcoholic fermentation. An immediate dominance of *S. cerevisiae* minimized off-flavors. However, the complexity provided by the indigenous yeast flora is also minimized. *Torulaspora delbrueckii* and *Kluyveromyces thermotolerans* have been shown to contribute with more complexity when added as starter cultures, although they are not able to complete the alcoholic fermentation as single strains. Combining *S. cerevisiae* with *T. delbrueckii* and/or *K. thermotolerans* has shown to secure the completion of the alcoholic fermentation, and provide flavor enhancement. Various yeast blends have been tested in Europe and United States for five years, and the fermentation data and flavor impact are shown to be consistent. Data show that *K. thermotolerans* and *T. delbrueckii* grew during the first part of the alcoholic fermentation alongside *S. cerevisiae*. They are eventually out-competed by *S. cerevisiae*, which continues to grow and complete the alcoholic fermentation. Data also show that *K. thermotolerans* and *T. delbrueckii* are present for an extended period during alcoholic fermentation when fermentation temperature is low. Sensory data indicate that the mixture of yeast species gives enhanced flavors, compared to alcoholic fermentation of pure strain *S. cerevisiae*.

### Laboratory Handling of Red Grapes to Estimate Wine Composition

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Winemakers sample grapes to predict wine quality. Researchers with hundreds of samples to analyze have time and labor restrictions during harvest that make it advantageous to store (either fresh or frozen) grape samples before analyses. This experiment compared storage and sample preparation methods to determine the most accurate and repeatable method to predict wine composition. Two storage methods for samples (fresh and frozen grapes) and two must preparation treatments (21°C and 71°C) on Cabernet franc (*Vitis vinifera*), Cabernet Sauvignon (*Vitis vinifera*), Chambourcin (interspecific hybrid), and Cynthiana (*Vitis aestivalis*) grapes were examined. Heating the must caused increased extraction of acids and some color components as compared to the wine. The use of the different must processing temperature may depend on the particular component of interest in the wine. In all cultivars, the titratable acidity, potassium levels, and red color

(520 nm) of the juice from the frozen grapes processed at 21°C had means that were most similar to the wine. Freezing the grapes before analysis provided the best approximation of composition in new red wine in the cultivars evaluated. However, if a particular component of the wine is of interest, then the storage method or processing temperature can be modified for peak estimation.

### **Increasing Iron and Calcium Stability in Wine and Other Beverages**

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Phytic acid is added to wine to chelate polyvalent iron and calcium cations. A calcium salt is added to coprecipitate the complex that subsequently is removed by filtration. This method effectively, inexpensively, and safely removes excessive levels of iron and calcium in wine, sparkling wine, and other beverages without changing the pH, color, or taste. Polyvalent metal cations such as iron and calcium when present in high concentration in wine and other beverages can adversely affect product quality. They may cause objectionable organoleptic properties including metallic taste, discoloration, and oxidative flavor changes as well as form hazes and cloudiness. Reducing heavy metal content in beverages, especially wines, has long been desirable. This method provides a novel means for selective removal of heavy metals, especially iron and calcium cations, from beverages such as wines and fruit juices. This method overcomes the problems of known methods; for example, it binds iron to the most complete extent possible, produces no toxicologically objectionable products even in the case of overclarification, and acts selectively on heavy metal ions.

### **Tannin and Polymeric Pigment in Fruit and Wine of Cabernet Sauvignon**

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The relationship between tannin in harvest fruit, tannin in wine after fermentation and aging, and tannin in the pomace was investigated using a protein precipitation assay. Cabernet Sauvignon fruit was collected at harvest from eight vineyard sites at two wineries in the Napa Valley with histories of giving wines with different amounts of tannin. After fermentation, pomace from the press was also collected and analyzed. The commercially produced wines were analyzed at pressing and after 30 and 70 days of aging. The percentage of tannin extracted was determined by comparing the total amount of tannin in the wine with the total amount in the fruit at harvest, resulting in a range of extractions from 32 to 75%. Harvest fruit tannin did not correlate with the amount of tannin in the resulting wine. During aging, almost all wines lost tannin and had a concomitant gain of nontannin iron reactive phenolics (NTIRP). These findings support the idea that tannins may be cleaved during aging. As the wines aged, the amount of NTIRP was strongly correlated with the formation of small polymeric pigment (SPP). Wines that had the most gain in SPP

were from alluvial, heavy soils, which may have contributed to differences in phenolic profiles of the resulting wines. The tannin measured in the pomace seeds often exceeded the amount measured in harvested seeds. There may have been tannin in the harvest seed that was covalently bound to the intracellular components and thus unextractable before fermentation.

### **General Viticulture Poster Session**

#### **Effects of Pruning, Girdling, and Gibberellic Acid Application at Bloom and Berry Set on Yield and Fruit Quality of Sweet Scarlet Table Grape Cultivar**

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Sweet Scarlet is a new midseason red seedless variety with a moderate Muscat flavor. Our objective was to determine the effect of pruning, girdling, and gibberellic acid (GA) on yield and fruit quality. Pruning treatments included spur-pruning on cordon-trained vines (42 nodes per vine) and a combination of spur- and cane-pruning to retain about 48 to 54 nodes on quadrilateral trained vines. Bloom application of GA at bloom did not significantly reduce berry set. There were differences in berry set between clusters on treated and untreated vines. GA applied at bloom had no significant effect on fruit bud differentiation in the following spring. Girdling and/or GA treatments were applied at berry set to evaluate their effect on berry size and fruit composition at harvest. Results collected during the 2003 and 2004 seasons showed that spur-pruned vines with fewer nodes produced a significantly lower yield than vines that had a combination of cane and spurs. Cane-pruned vines had a lower percent budbreak and cluster to shoot ratio than spur-pruned vines. Yield in 2004 was reduced by the GA treatments applied in the preceding two seasons (2002 and 2003), which also affected the results of the pruning trials. Girdling or GA alone caused a slight increase in berry weight or size (~5%). However, girdling plus GA at 20 or 40 mg/L significantly increased berry weight and berry size (length and diameter). The largest berries were produced on vines that were girdled and sprayed with GA at 40 mg/L at berry set. This treatment also delayed color development and significantly lowered titratable acidity. Increase in berry size may have also been influenced by the relatively lower yield produced in 2004 on GA-treated vines.

#### **Response of Merlot Grapevines to Drip and Sprinkler Irrigation in the Okanagan Valley**

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The effects of sprinkler versus drip irrigation on vine development and fruit maturation were explored over two years in a Merlot vineyard with a sandy soil in the Okanagan Valley in British Columbia. Sprinkler and drip-irrigation treatments were applied to main plots, and cluster thinning treatments were applied to subplots in a spit-plot design. Irrigation was applied when the soil moisture content at 15 to 30



cm depth was depleted to less than 10%. Sufficient water was applied to wet the soil profile to below 45 cm. In the first and second years, sprinkler irrigation was applied 23 and 18 times, respectively, and drip irrigation was applied 31 and 21 times. During summer, stomatal conductance and photosynthesis rates were lower in drip- than in sprinkler-irrigated vines, regardless of the soil moisture content under the vines. Compared with sprinkler irrigation, drip irrigation reduced vine vigor, berry weight, and yield in the first year and reduced cluster weight and yield in nonthinned vines in the second year. In vines carrying similar yields, fruit matured earlier and had lower acidity under drip than under sprinkler irrigation. Monitored ambient temperatures revealed that daily maximum temperatures and GDD accumulation were higher under drip than under sprinkler irrigation, which accounted for the differences in fruit maturation.

### Petiolar Analysis, pH, and Soil Management of a Vineyard with Pinot noir, Cabernet Sauvignon, and Merlot

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An analysis was conducted in a vineyard newly planted with Pinot noir, Cabernet Sauvignon, and Merlot, located in a valley 200 m above sea level in Itaara, Rio Grande do Sul, southern Brazil. Soil analysis followed by preplant soil amendment and fertilizer additions resulted in unbalanced plant nutrition. Must and wine had high pH and needed the addition of tartaric acid to hold pH below acceptable levels. Potassium and several other elements were above or below acceptable established values. Petiolar analysis (including N, K, P, Ca, Mg, Mn, Fe, Cu, B, Na, Cl, and Zn) was conducted. Samples of 100 petioles were taken from the opposite side of inflorescence at full bloom and 30 days later. Samples were dried, crushed, digested, and analyzed. Nitrogen was analyzed by the Kjeldahl method and the other chemical elements by spectrophotometry (VIS/UV), flame photometry, and atomic spectrophotometry. Two years after the first sample analysis, a careful program of fertilization has brought the soil under better control. Field data (temperature, precipitation, heat summation), Brix, total acids, and pH were also followed the last two seasons. Results show that must and wine pH and other chemical elements are approaching ideal numbers. The analysis will continue for several years.

### Characterization of Xylem Anatomical Structures and the Effect of Xylem Sap from *Vitis* on Pierce's Disease

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This study tests the hypothesis that Pierce's disease (PD) resistance is due to the presence of antimicrobial compounds expressed in the xylem sap that suppress *Xylella fastidiosa* (Xf) and/or to the anatomical features of the xylem, such as pit membranes that restrict Xf mobility in xylem. Grape species and cultivars were selected for study based on suspected or confirmed differences in the way they resist PD. To evaluate

whether PD resistance is associated with xylem structure, a PD-resistant stem was used as an intergraft bridge within a PD-susceptible grape. This intergrafting method allows examination of how the pathogen moves across resistant xylem tissue. ELISA was used to quantify Xf movement. Scanning electron microscopy was used to characterize xylem structure. An in vitro bioassay was developed to examine whether xylem sap collected from PD-resistant grapes is antimicrobial by its affect on biofilm formation or suppression of Xf growth. The preliminary bioassay results suggest that xylem sap from some PD-resistant grapes, such as *Vitis shuttleworthii*, hybrid 8909-15 and 8909-17 (*V. rupestris* x *V. arizonica*), limits biofilm formation and Xf growth when experiments were compared with sap from the highly susceptible Chardonnay.

### Relation of Sucrose Transport to Electrical Potential Difference between Petiole and Berry

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Electrically charged sucrose is commonly employed by plants to facilitate movement through cell membranes. This study addresses the question of whether this same mechanism is employed to move sucrose from the leaf to the berry. Electrochemical sensors were implanted in 15 petioles and berries in a block of Syrah and a block of Pinot noir vines in Monterey County, California. Measurements were made every half hour of the electrical potential of the petiole and berry from preveraison to harvest as well as cluster count, cluster weight, and Brix. Data analysis indicated electrical potential difference between petiole and berry follows a double sigmoid pattern which relates to sucrose movement. This relation can be explained with a quantitative electro/osmotic model which takes into account the magnitude of the sucrose source, the resistance to transport in the pathway between source and sink, and the number of berries on the shoot. Conceptually, the model center around the following relation: (source strength) \* (pathway resistance between leaf and berry) = (force required to transport the sucrose between leaf and berry).

### Transcription of Flavonoid 3'- and 3',5'-Hydroxylase Genes Controls Grape Anthocyanin Composition

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Anthocyanin composition is an important character of grape cultivars. The anthocyanin composition of grape is believed to be controlled by the ratio of flavonoid 3'-hydroxylase (F3'H) and flavonoid 3',5'-hydroxylase (F3'5'H) activities as well as anthocyanin methyltransferase activity. In this study, mRNA levels of F3'H and F3'5'H genes were determined to investigate whether the transcription of these genes controls the anthocyanin composition of grapes. At first, DNA sequences of grape F3'H and F3'5'H genes were obtained based on expressed sequence tag data of grapes. Southern hybridization showed that haploid grape genome had probably one copy of F3'5'H gene and two copies of F3'H gene. Real-time RT-PCR analysis showed that mRNA concentration of F3'5'H was higher than that of F3'H in the berry skins of Cabernet Sauvignon and Dornfelder, while the berry skin of Muscat

Hamburg showed the opposite result. The berry skins of Cabernet Sauvignon and Dornfelder contained malvidin-3-glucoside, which has two methoxyls in the 3' and 5' positions, and its acetic acid and *p*-coumaric acid esters as main anthocyanins, while the berry skins of Muscat Hamburg contained mainly peonidin-3-glucoside, which has a methoxyl in the 3' position and its *p*-coumaric acid ester. Thus, the mRNA levels of F3'H and F3'5'H in the berry skins agreed with the anthocyanin composition, indicating that the transcription of F3'H and F3'5'H genes plays an important role in controlling anthocyanin composition in grape skins.

### **Viticultural Performance of Syrah Grapevines on New USDA-ARS Rootstocks for Winegrape Production in the San Joaquin Valley**

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The viticultural performance of Syrah grapevines grafted on USDA-ARS rootstock selections 10-23B, 10-17A, and 6-19B was evaluated in comparison to commercial rootstocks Freedom, Teleki 5C, 1103 Paulsen, and self-rooted vines in the San Joaquin Valley, CA. The vineyard was planted in north-south rows on 244 x 366 cm (vine by row) spacing in 1995, trained to a vertical bilateral cordon in 1996, and drip irrigated. The trellis system consisted of a cordon wire mounted at 132 cm and a single foliage wire mounted at 163 cm. Soil type was Hanford sandy loam, not fumigated before planting. The experiment was designed as a randomized complete block with seven treatments and four replications of five vines. Vines on 1103 Paulsen, Freedom, and Teleki 5C performed comparably, except that 1103 Paulsen produced must of higher Brix and wine of greater color. Highest petiole nitrate content was found on 1103 Paulsen, which may explain the higher yield and greater vigor. Self-rooted vines had lower petiole nitrate and Mn content and supported higher population of Lesion PV nematodes. Rootstocks 10-17A and 10-23B performed similarly and were comparable to commercial rootstocks. Noticeable differences included higher wine TA, total phenolics, and color in vines on 10-23B in 2000. Vines on 10-23B had lower petiole Mn content in 1999. Fruit maturity in vines on 10-17A and 10-23B was similar to commercial rootstocks and self-rooted vines. USDA-ARS selection 10-23B was found susceptible to phylloxera. Vines on 6-19B showed the lowest yield, lowest vigor, highest light penetration into fruiting zone, highest must Brix and color, lowest must TA, lowest petiole nitrate and Mn content, and highest petiole K content. The lowest petiole nitrate content in vines on 6-19B may explain the low yield and vigor. Vines on 6-19B were the most susceptible to water stress.

### **Viticultural Performance of Thompson Seedless Grapevines on New USDA-ARS Rootstocks for Raisin Production in the San Joaquin Valley**

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The viticultural performance of Thompson Seedless grapevines grafted on USDA-ARS rootstock selections 10-23B, 10-

17A, and 6-19B was evaluated for raisin production in comparison to Harmony and self-rooted vines in the San Joaquin Valley, CA. The vineyard was planted in east-west rows on 213 x 366 cm (vine by row) spacing in 1995, head trained in 1996, cane pruned, and drip irrigated. The trellis system was a 61 cm cross-arm mounted at 183 cm from the soil surface. Soil type was Hanford sandy loam, fumigated with methyl bromide in 1994. The experiment was designed as a randomized complete block with five treatments and five replications of five vines. In fumigated soils, yield of self-rooted vines was similar to vines on Harmony but with much lower vine vigor. Vines on Harmony produced smaller berries. Petiole K content was lower but Mg content higher in self-rooted vines than vines on Harmony. Fruit in self-rooted vines had lower Brix and pH. Vines on USDA-ARS selection 10-17A had lower yield, smaller berries, but similar vigor compared to vines on Harmony. The lower yield was due to fewer clusters per vine on this rootstock selection. Vines on 10-17A had lower P and higher Mn content. Yield and petiole mineral nutrients were similar between 10-23B and Harmony. USDA-ARS selection 10-23B was found susceptible to phylloxera. Vines on 6-19B had similar yield but much lower vigor compared to vines on other rootstocks. Vigor of vines on 6-19B was similar to self-rooted vines. Petiole nitrate content was much lower in vines on 6-19B in 1999; however, it increased to a comparable level to vines on Harmony during subsequent years.

### **Effect of Cane Severance Date on Soluble Solids, Quality, and Yield of Dry-on-the-Vine Raisins**

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For dry-on-the-vine (DOV) raisins, cane severance must be late enough that fruits amass the soluble solids needed for good-quality raisins but early enough that the fruits dry sufficiently. Generally, grapegrowers sever canes when berry soluble solids reach an average of 20 Brix, an optimal level for tray-dried fruits. However, anecdotal evidence suggests that berries of similar soluble solids achieve higher raisin grades when dried on the vine than on trays. If so, then the DOV process could be started earlier to increase the likelihood of drying without reducing quality. Therefore, we determined the effect of different cane severance dates on berry soluble solids, raisin quality, and yield. Fruit-bearing canes of Thompson Seedless grapevines (*Vitis vinifera* L.) were severed on 22 July, 5 August, 16 August, or 2 September 2004, when berry soluble solids averaged 15.4, 17.5, 20.2, and 21.7 Brix, respectively. There was a curvilinear increase in the proportion of raisins graded "B and better" across a range of soluble solids, from 14 to 24 Brix. The proportion of B and better raisins from the first three cane severance dates was 40%, 56%, and 77%, respectively; an increase of almost 30% between each date. Raisins from the last two severance dates had similar grades. The first severance date also decreased yield by 25% compared to the three subsequent dates. Unfortunately, only the first two severance dates were early enough to dry the raisins sufficiently. Thus, early cane severance, to ensure drying, may unacceptably reduce raisin quality and yield.

## Options for Weed Management in Vineyards

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Weed management in both young and established vineyards (*Vitis vinifera*) is important to ensure proper growth and seasonal development. Many growers have reduced or eliminated weed management to conserve financial resources. This may be detrimental as weeds compete for water, nutrients, sunlight, and space, interfere with effective harvest, and physically reduce yield. Weed resistance is occurring with commonly used herbicides in vineyards such as marestalk (*Conyza canadensis*) resistance to glyphosate. There can also be problems with some residual materials because of application timing restrictions. Studies were conducted from 2001 through 2004 to evaluate and compare efficacy and crop safety of new herbicides to standard materials for weed management in vineyards. Trials were established using a randomized complete block design. Treatments were applied with CO<sub>2</sub> backpack sprayers delivering 20 gallons of spray solution at 40 psi. Visual evaluations were conducted throughout the year for both weed control and crop injury. Many of the new materials tested have shown efficacy against a number of problem weeds without evidence of crop injury.

## Pruning and Training System Effects on Yield, Time of Harvest, and Fruit Quality of Concord Grapevines in Michigan

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Mature Concord grapevines in a commercial vineyard were subjected to eight treatments from 1997 to 2001: balanced pruned (20+20/.45 kg cane weight, maximum 80 nodes) (BP); BP shoot positioned (SP) ≈1200 growing degree days F; 90 nodes; 130 nodes; simulated hedging; minimal pruned (MP); MP SP; and MP fruit thinned to a single basal cluster before veraison. The SP treatment had no significant effect on any parameters. Nonthinned MP vines consistently had the highest yields; however, they sometimes failed to reach minimum soluble solids levels until after the commercial harvest season. Balanced-pruned vines had relatively low yields of early-ripening fruit. Minimal-pruned vines had moderate yields of good quality, early-ripening fruit. The 90 node, 130 node, and hedged treatments consistently produced acceptable fruit during the commercial harvest period, although the latter two treatments showed a possible tendency of biennial bearing.

## Fate of Vine Mealybug *Planococcus ficus* (Signoret) in Winery Waste

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Vine mealybug, *Planococcus ficus* (Signoret), is an exotic pest in California that has been found in vineyards in several growing regions. Sanitation practices are required to prevent further movement and reduce its incidence. The waste from infested fruit may remain contaminated with viable in-

sects after pressing. There is a subsequent risk that vines may become infested when winery waste (pomace and stems) is spread as mulch in row middles. Infested clusters were added to 6- and 12-ton press loads of Grenache and Chardonnay grapes. Into the press were placed mesh bags that contained single infested units of whole cluster, rachis, or detached berries + rachis. Press regimen varied for each load, and pressures reached 1.8 and 2.0 bars in 4.0 and 3.5 hour periods, respectively. Postpress, insects survived in all bags and severity of infestation ranged from 229 (4.4%) to 1196 insects (23.1%) per bag. Vine mealybug mortality in winery waste was evaluated by placing mesh bags containing infested stems at two depths inside four covered and four uncovered piles of pomace and stems in the Alexander Valley AVA. A sensor recorded temperatures continuously at each depth in all piles over a four-week period. A stem bag was removed weekly from each depth. Mortality of vine mealybug ranged from 61 to nearly 100% after one week and was a function of coverage and stem content. Insect mortality ranged between 99.99 to 100% for stems that remained in the piles for four weeks. Temperature was inversely related to percent stems ( $r^2 = 65.2$ ).

## Effects of Site Conditions on Pinot noir Color in the Okanagan and Similkameen Valleys

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The Okanagan and Similkameen Valleys in British Columbia contain substantial regional diversity in soil types and climatic conditions. To understand the relative impacts of site conditions and management practices on Pinot noir color, we examined the compositional quality of fruit from 40 vineyards in six geographic regions over three years. Fruit was sampled from two to six vines at two locations within each vineyard. Vine vigor, fruit microclimate, and nitrogen status were determined for each vine. Vine vigor and the factors that contributed to vigor, such as leaf nitrogen, were negatively correlated with anthocyanin concentration in the berries. Compared with large berries, small berries had a high proportion of skin tissue and the skin tissue contained a high concentration of anthocyanins. Berry size and skin % dry matter were not correlated but berries with either one or both characteristics had high anthocyanin concentrations. Growers that attained a high level of color in Pinot noir generally produced less vigorous vines with small berries and high skin % dry matter while using different management styles and site conditions.

## Sugar Transport and Regulation in *Vitis vinifera* Suspension Cultured Cells

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Sugar transport is of utmost importance for crop productivity. The most dramatic change in berry development occurs as the fruit enters into the ripening phase. Berries, which were small, firm, and acidic with little sugar become larger, softer, sweet, and strongly flavored. Up to now, several genes (*VvHT*:



*Vitis vinifera* hexose transporters) have been cloned from ripening berries and display different patterns of expression during ripening. In this work, *V. vinifera* heterotrophic suspension-cell cultures obtained from berry flesh cells were used as a model system to study sugar transport and its regulation. Sucrose-grown cells hydrolyzed the disaccharide within four days, and thereafter glucose was depleted from the medium before fructose. Cells were able to transport D- $^{14}\text{C}$ glucose according to Michaelis-Menten kinetics ( $K_m$ , 40 mM glucose) plus a first-order kinetics ( $k_d$ , 0.08 mL/min/mg dry wt) and  $\text{H}^+$ -dependent transport accounts for the saturating component. Beyond its importance as nutrient, glucose also behaved as a signal molecule tightly regulating carrier activity, VvHT1 protein amount, and VvHT1 transcripts. In addition to a repressing effect at high concentrations, the monosaccharide was also shown to be required for induction of sugar transport. This study deepened our understanding of berry ripening mechanisms and highlighted the complex pattern of regulation of hexose transport in plant cells.

#### Industrial Poster Session

##### Efficacy of Lysozyme in Controlling Histamine Production by *Lactobacillus hilgardii* during Winemaking

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Biogenic amines are reported to be produced by lactic acid bacteria (LAB) in wine and to be a causative agent of an intolerance to wine consumption experienced by some consumers. The levels of biogenic amines such as histamine in wine are closely scrutinized, with maximum legal limits imposed in certain countries. The objective of this study was to investigate the efficacy of lysozyme in controlling the growth and the histamine production of a well-studied histamine-producing LAB culture. The *Lactobacillus hilgardii* strain was inoculated into sterile grape juice at  $1 \times 10^7$  cfu/mL with various levels of lysozyme: 0 (control), 125, 250, and 500 mg/L. Alcoholic fermentation (AF) was carried out by commercial wine yeast. During AF, LAB, yeast, and acetic acid bacteria were enumerated on specific media. Sugar, malic acid, acetic acid, and ethanol contents were measured enzymatically. Biogenic amines were analyzed using HPLC equipped with a fluorescent detector and a RP (C18) column, after precolumn derivatization with *o*-phthalaldehyde. Under the experimental conditions, a 7-log cell reduction was achieved with the addition of 125, 250, and 500 mg/L lysozyme. On day seven, the histamine content in the control reached 35 mg/L; in the lysozyme-treated wines, the histamine level remained below 2.0 mg/L. Results indicate that, when used preventively at the beginning of AF, lysozyme is able to inhibit the growth of the wine-spoilage LAB and subsequently to prevent the formation of histamine by the spoilage bacteria.

##### Crop Water Stress Index Measured by the Sap Flow Method on Vines Correlates with Deficit Irrigation

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The objective of the study was to evaluate a transpiration measurement method, which collects and processes data to measure crop water stress index (CWSI). Transpiration stress has been evaluated over many years with other methods such as infrared leaf temperature analysis and leaf water potential. Our study compared irrigation deficit and crop stress to determine whether transpiration stress is controllable. Initially we collected sufficient sap flow data from the Cuesta Ridge Vineyard to compute a crop coefficient,  $K_c$ , to relate evapotranspiration data to the crop water flux over a six-week period. Weather and evapotranspiration potential,  $\text{ET}_0$ , was collected from the nearest CIMIS weather data station. Two different irrigation schedules yielded the same crop coefficient under well-watered conditions. In effect, during well-watered conditions, transpiration correlated to  $\text{ET}_0$  at a constant ratio throughout the measurement period. The next step was to convert the sap flow results to crop water stress and compare the results day by day to the irrigation deficit. A maximum evapotranspiration ( $\text{ET}_m$ ) was also calculated for all days by indexing the  $\text{ET}_0$  for the period with crop coefficient. The difference between the  $\text{ET}_m$  and the actual transpiration ( $\text{ET}_a$ ) was used to compute CWSI on a daily integration of all hourly data. The transpiration stress index was calculated daily and increased as water depleted from the soil. A direct correlation of lower volumetric water content and irrigation deficit to increased transpiration stress was shown. Sap flow measurements quantified transpiration stress and how the sap flow in vines related to expected transpiration derived from  $\text{ET}_0$  in well-watered and stressed conditions. The average transpiration stress, CWSI, increased from 0.25 to 0.30 (+ 5%) after the irrigation deficit was increased from 30 to 40% over a four-week period.

#### Student Enology Poster Session

##### Cell Membrane Damage Induced by Phenolic Acids on Wine Lactic Acid Bacteria

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Phenol-derived compounds are known to have membrane-active properties against microorganisms causing leakage of cell constituents including proteins, nucleic acids, ATP, and inorganic ions (such as potassium or phosphate). Phenolic (hydroxybenzoic and hydroxycinnamic) acids are components of the highly complex phenolic composition of wines. In this work, several wine-occurring phenolic acids, that were previously found to affect the growth and the survival of certain wine lactic acid bacteria, were tested for their effects on the cell membrane of these same strains. Selected strains of *Lactobacillus hilgardii* and *Oenococcus oeni* were cultivated to late exponential phase and then centrifuged, washed, and resuspended in buffer solution. Phenolic acids were added to

the cell suspensions to induce chemical stress. Potassium leakage from cells was measured by determining the extracellular potassium concentration of the medium using a potassium ion-sensitive electrode. A combined glass electrode was used to monitor pH changes. Phosphate efflux was determined using a flow injection analysis (FIA) system with spectrophotometric detection. Samples were collected at regular intervals, microfiltered, and injected in the FIA system. Results indicate that *p*-coumaric acid had the strongest effect of all phenolic acids, causing rapid potassium and phosphate leakages from cells after exposure to this compound. Generally, hydroxycinnamic acids induced faster leakage rates than hydroxybenzoic acids, which could be related to their higher lyophobic character. These results agree with previous growth and survival experiments. The differences of phenolic acids toxicity toward lactic acid bacteria seem to be related to their effects on membrane permeability.

### Mutational Analysis of Genes Involved in Malolactic Fermentation in *Oenococcus oeni*

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In *oenococci*, the malolactic conversion is linked to the *mle* locus (*mleRSP*); however, a mutational analysis of these genes in situ has yet to be achieved. Moreover, few oenococcal genes other than *mleRSP* have been linked to the malolactic conversion. To address this, we employed an insertional mutagenesis strategy using the conjugative transposon Tn925 and identified transconjugants with impaired malolactic fermentation (MLF) on colorimetric plates. The mutants were subsequently checked for efficiency of MLF in simple conversion assays and resting cell ML conversion assays and several integrants with impaired MLF capacity were confirmed. To identify the gene(s) involved, the site of Tn925 insertion for a sample integrant (mutant 10) was sequenced and shown to be immediately upstream of a gene with homology to ion transporters (termed *mleY*). To confirm linkage to MLF, we identified and disrupted the same gene in *Lactococcus lactis*, a genetically pliable host that also performs a MLF. The lactococcal *mleY* mutant did not show altered growth or obvious impairment of MLF; however, resting cell ML conversion assays did show slight differences. Complementation of the lactococcal *mleY* mutant with either the *O. oeni* or *L. lactis* *mleY* gene *in trans* partially restored the WT phenotype, suggesting some, albeit minimal, linkage to the MLF. Efforts to characterize additional oenococcal Tn925 mutants are underway. In this work we have demonstrated an approach to use other, genetically pliable, lactic acid bacteria to identify and characterize genes in *oenococci* that are linked to MLF.

### Defining the Genetic Basis of Hydrogen Sulfide Production

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Production of hydrogen sulfide (H<sub>2</sub>S) by *Saccharomyces cerevisiae* is undesirable. Different factors have been asso-

ciated with production of volatile sulfur compounds, but production levels vary across strains in response to these conditions, suggesting that differences in internal enzyme regulation and activity affect H<sub>2</sub>S production. A screen of the yeast deletion strain set, comprised of 4,827 mutants, on BiGGY agar was conducted to identify genes responsible for hydrogen sulfide production as well as genes leading to dark colony color. This set includes all known nonessential genes in the *Saccharomyces* genome. BiGGY agar is a differential medium that evaluates relative production of sulfide. Sulfide generated by the yeast colony forms a black precipitate and the intensity of color is dependent on the amount of sulfide produced. The mutants were classified into six different color groups: white (4), light tan (257), tan (4,476), light brown (61), brown (28), and black (1). Wine strains display colors ranging from white to brown. The light brown and brown colonies found by the screen were subjected to a second screen in synthetic juice media as well as Pinot noir juice to evaluate production of hydrogen sulfide. Fourteen of the 100 darker staining mutants that produced H<sub>2</sub>S are currently being crossed to wild-type strains to define the effect of the mutation on H<sub>2</sub>S production. Five of 14 mutations are in the methionine synthesis or transport pathway, with others involved in various pathways such as amino acid or nitrogen compound transport or utilization.

### Effect of Cheese and Wine Pairing on Red Wine Flavor Profile

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The study assessed how the flavor perception of red wine was influenced by pairing with a variety of cheeses. The study was performed by descriptive sensory analysis. A panel of 11 trained judges evaluated the flavor of eight wines of four different varieties (Cabernet Sauvignon, Merlot, Pinot noir, and Syrah) before and after tasting cheese. Eight cheeses, including two soft cheeses (Mozzarella and Teleme), two medium-hard cheese (New York Cheddar and Vermont Cheddar), two hard cheeses (Emmental and Gruyère), and two blue cheeses (Gorgonzola and Roquefort), were selected for the pairing. The results obtained by descriptive analysis showed that the paired cheese has a significant effect on red wine flavor. Attributes such as astringency, bell pepper, and oak flavor diminished significantly after tasting cheese compared to the unpaired wine. On the other hand, butter aroma was significantly enhanced for wines paired with cheddar cheeses and perceived bitterness increased for wines paired with blue cheeses. It was also found that the effect of any given cheese is equivalent for all wines; in other words, there was no significant wine-cheese interaction effect.

### Rapid Automated Analysis for Phenols during Wine Processing

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A new automated method for measuring wine phenols using segmented continuous flow analysis has recently been

developed. The advantages of this method include greater precision and accuracy in measuring total phenolic content in terms of gallic acid equivalents versus traditional wet chemistry approaches. Additionally, the method enables a high sample throughput with a capacity of 50 samples per hour. This method has been used to monitor the phenolic development of five different wine varieties from harvest through the entire winemaking process.

### Student Viticulture Poster Session

#### Strain Differences in *Xylella fastidiosa* Observed Using Indirect Immunofluorescence

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The gram-negative, plant pathogen *Xylella fastidiosa* (Xf) is prevalent in many native plants located in the Gulf Coast of Texas. As a result, Pierce's disease (PD) can be a significant economic loss for Texas vineyards, particularly those in the southeast. Using indirect immunofluorescence to visualize Xf in sap of both plant reservoirs and infected vines, it became apparent that there were observable strain differences under the fluorescent scope using standard ELISA antibody. There were consistent strain differences in bacterial length and brightness (over time and irrespective of experimental variables such as media and temperature). A preliminary evaluation of cell lengths between two grape strains found one strain to be consistently and significantly longer than another ( $p = 0.0004$ ). In addition to variation in size and brightness, some strains consistently show a localized immunofluorescence. This suggests that the composition, concentration, or distribution of the cellular protein specific for the primary antibody differs between strains. The localization of the signal suggests the protein may be related to pili distribution, which are concentrated at one end in some electron micrographs of Xf. The drastic difference in brightness between strains also indicates that antibody-related detection methods (ELISA) will not be appropriate for quantifying bacterial levels across strains. We are currently comparing immunofluorescence differences in the Texas strains to genetic relatedness of strains using gyrase B gene phylogenetics.

#### Effect of Irrigation, Tillage, and Nitrogen Supplementation on the Formation of Sulfur Compounds in Pinot noir Wines

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Volatile sulfur compounds in two vintages (2000 and 2001) of *Vitis vinifera* cv. Pinot noir wines from different viticultural practices were studied using solid-phase microextraction (SPME)–gas chromatography (GC)–pulsed flame photometric detection (PFPD). Wines were made from two field blocks of 12 factorial combinations of irrigation (dry or irrigated), tillage (tilled or not tilled), and fertilization (none, foliar nitrogen supplementation, or soil applied nitrogen). A total of 11 sulfur compounds were investigated. Analysis of variance

(ANOVA) was performed using S-PLUS. Results showed that nitrogen fertilization was associated with the concentrations of hydrogen sulfide and methanethiol. Both foliar nitrogen supplementation and soil applied nitrogen increased the concentration of hydrogen sulfide ( $p < 0.05$ ) and methanethiol ( $p < 0.01$ ) in final wines in both vintages. Irrigation and soil tillage had no impact on the formation of hydrogen sulfide and methanethiol. However, irrigation significantly increased the methyl thioacetate and ethyl thioacetate in the 2000 vintage ( $p < 0.05$ ) but not in 2001. Tilling treatments generated significantly higher concentrations of dimethyl sulfide in the 2001 vintage than those not tilled ( $p < 0.05$ ).

#### Field-Sampling Technique and Population Evaluation of Leafhoppers in Winegrape Vineyards

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The composition of leafhopper populations and the validation of sampling methods by which field data are collected are important to ensure the applicability of vineyard pest management decisions. In this study, commonly used field-sampling techniques for leafhopper presence in winegrape vineyards of south central Washington State were tested for accuracy. In addition, the species and lifecycle population distribution of leafhoppers were investigated during the early growing season of 2004. The leafhopper population at individual sites was dominated by either the Western Grape Leafhopper species (*Erythroneura elegantula*) or the Virginia Creeper Leafhopper species (*Erythroneura ziczac*). Percent infestation via presence/absence sampling and leafhopper average per leaf via hand-lens use were compared to an absolute method of microscope investigation of leaf surfaces to determine the precision of such sampling methods. The data was analyzed via ANOVA regression and scatter grams to determine the efficiency of each technique. In general, both field-sampling methods were solidly correlated to the absolute method ( $R > 0.73$ ), although the presence/absence sampling technique was the most accurate ( $R > 0.79$ ). This indicates that percent infestation calculations may be more efficient than hand-lens counts for immediate use, although speciation and egg identification are not possible with this method. Furthermore, both field method investigations yielded accurate linear prediction models that may be used in future field applications to make accurate appraisals of leafhopper populations in the vineyard, thus reducing unnecessary pesticide use and preserving wine quality.

#### Comparison of Three Potential Insect Vectors of Pierce's Disease across Eight Texas Vineyards

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The glassy-winged sharpshooter (GWSS) (*Homalodisca coagulata*) is an efficient vector of the plant pathogen *Xylella fastidiosa* (Xf) which causes Pierce's disease (PD) in grapevines. Although a recent threat in California, recent genetic studies suggest the GWSS is native to Texas. For these rea-



sons, study of the primary GWSS infecting vineyards across Texas and evaluation of the Xf level within the insects is critical for understanding the ecology of the vector and epidemiology of PD. Three insect species with the highest trap frequencies in Texas, *H. coagulata*, *Graphocephala versuta*, and the spittle bug, *Clastoptera xantoccephala*, were collected on sticky traps from eight Texas vineyards during two weeks in July 2004. Several hundred insects were crushed and ELISA tests performed on both trapped and guaranteed clean lab insects. Frequency of GWSS species varied with location as did the percentage of infected GWSS. Evaluation of environmental variables suggests insects increase with poor weed control. Data also indicate that all three species can reach high numbers in the vineyard and all three species are capable of carrying high Xf levels (4X negative insects).

### Color and Tannin Profiles as Influenced by Rootstocks

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Rootstocks can control pests, vigor, yield, and fruit composition, playing a fundamental role in the overall success of vineyard operations. This trial was planted at the Oregon State University research vineyard in 1997. Merlot was grafted to nine different rootstocks and compared to ungrafted vines. Pinot noir was grafted to 10 additional rootstocks. Vine physiological performance, fruit yield, and composition were evaluated. Pinot noir wines were made from six selected rootstocks. Both Pinot noir and Merlot grafted to Riparia Gloire had overall higher soluble solids, higher pH levels, and lower acidity, probably due to the very low crop yields these vines produced. 5BB and 420A rootstocks generated the highest yields with lower pH levels and higher acidity of the juice. Ungrafted Pinot noir and Merlot vines and those grafted to 3309C had lower soluble solids in the juice when compared to the other rootstocks. Wines originated from Pinot noir grafted to 420A had the highest color intensity and lowest hue. The highest hue was found in wines from Riparia Gloire and 3309C, possibly indicating a premature aging. Proanthocyanidin and anthocyanin profile analysis in skin and seed extracts have not yet been completed. Results will provide a better understanding of the role of rootstocks in determining fruit and wine chemistry.

### Rootstocks Use Different Drought Avoidance Strategies

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Rootstock selection helps with problems such as *phylloxera*, nematodes, or site difficulties. Rootstocks can also control

vigor, yield, and fruit composition. This trial was planted in 1997 at the Oregon State University research vineyard, located in Oregon's Willamette Valley. Pinot noir was grafted to 19 different rootstocks and compared to ungrafted vines in a completely randomized block design. Vines were balanced pruned to 30 buds/kg of pruning wood and did not receive any supplemental irrigation. Physiological measurements included gas-exchange, chlorophyll content, and vegetative growth. Vine water status was assessed by midday stem water potential measurements. Stem water potential was revealed as a powerful whole plant stress indicator, being closely correlated with stomatal conductance, photosynthesis, and transpiration. A close relationship between stem water potential and berry weight was also found. Berries tended to be smaller in vines with lower water potentials, which was independent of the vigor imposed by the different rootstocks. Rootstocks use different strategies to avoid drought. Some restrict canopy development (Riparia Gloire, Börner, 44-53M, 3309C); others have a lower drought stress threshold (140Ru, 99R, 5C); while some vigorous rootstocks are able to maintain nonstressing water status (1103-P, 125AA, 5BB, 1616C). It is therefore important to adjust the start of irrigation and the amount of water to the rootstock used.

### Influence of Mother Vine Canopy Light and Fertilization on Adventitious Root Formation in Grapevine Rootstocks

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The rooting ability of grapevine rootstock cuttings was tested against the growing conditions of the rootstock mother vines. Mother vines were either trained to a simple trellis or allowed to grow freely along the ground as a mat of foliage. Vines were either fertilized with nitrogen or left unfertilized. Trellised vines had previously been shown to have greater canopy light penetration than the matted vines. Three rootstocks (420A, 101-14, and 110R) were used in this study. Rooting ability was measured as the number and weight of adventitious roots that grew from benchgrafts of the rootstocks over an extended six-week callusing period. 101-14 formed significantly more roots with a significantly larger root mass than either 110R or 420A across all mother vines treatments. 110R and 420A formed more roots and larger root masses when mother vines had been trellised than when mother vines had been matted, although differences were not significant. 101-14 cuttings produced significantly more roots when mother vines were not fertilized, but these roots were larger when the mother vines were fertilized. Nitrogen fertilization increased the number of roots formed by 420A and 110R and increased the root masses of 420A as well, although neither of these differences were significant.