Abstracts

Papers and Posters Presented at the ASEV 55th Annual Meeting
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Wine Composition

Effects of Closure Type on Wine Quality Characteristics

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The impact of different closure types on the analytical and sensory properties of wines has been the subject of much conjecture, but little has been quantified. This study explores the effects of time on commercial Chardonnay and Merlot wines stored with five different closures: natural cork, two synthetic cork stopper alternatives, and two screw-cap closure liners. We commenced with the hypothesis that there would be no discernable difference among closure treatments. Descriptive analysis was conducted at the winery with an expert panel of production staff members in three series of tastings over 12 months. The final session, December 2003, represented 30 months of bottle aging. After 30 months in-bottle, natural corks preserved the wines well but resulted in lower fruit and higher cork-associated characteristics (such as TCA, earth, dirt, mustiness) when compared to screw-cap closed wines. The synthetic closures resulted in clean wines that were not as well preserved, were showing aged character, and had an overall lower level of fruit. The screw-cap-closed wines were clean and well preserved, with a statistically significant higher level of fruit aroma and taste in both wines. This sensory data was reinforced with multiple replicate analytical results showing that the synthetic closures resulted in gradual oxidation while the screw-cap closures protected the wines. The natural cork closure did show relatively good SO_2 and CO_2 retention and no evidence of oxidation or browning.

Use of Ferric Chloride for the Measurement of Total Phenolics in Red and White Wines

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Phenolics are important in wines because they are responsible for color, bitterness, and astringency. Total phenolics in wines is traditionally measured by the Folin-Ciocalteu (FC) method. The purpose of this work was to evaluate ferric chloride for use as a colorimetric reagent to measure total phenolics in wines. We tested representatives from the most abundant classes of phenolics found in grapes and wines for their reactivity with ferric chloride. We found that caffeic acid, caftaric acid, catechins, quercetin, kaempherol, and gallic acid were all iron reactive. However, anthocyanins, the second most abundant class of phenolics in red grapes and wines, were not detected with ferric chloride. Assessment of the different phenolic classes with the ferric chloride reagent demonstrated that all phenolics containing vicinal dihydroxyls gave a positive reaction, with the exception of the flavonol kaempherol. Twenty-six wines were analyzed by both FC and ferric chloride methods to determine how the two procedures compared for measuring total phenolics found in wines. Comparison between the FC and ferric chloride methods yielded a high correlation ($r^2 = 0.92$, $n = 26$) indicating that ferric chloride is a suitable reagent for all phenolics in wine except anthocyanins. Thus, the method should be deemed a measurement of total iron reactive phenolics. When combined with a simple assay for anthocyanins, tannins, and polymeric pigments, this procedure provides a convenient assessment of all of the functional classes of phenolics in grapes and wines.

Effect of Vine Microclimate on Norisoprenoid Formation in Carbernet Sauvignon Grapes and Wines

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The effect of vine microclimate on the concentrations of C13-norisoprenoids in Carbernet Sauvignon grapes and wines was investigated. Six vine treatments were studied: (1) exterial clusters tucked into the canopy (ECTC), (2) control (no manipulation), (3) primary leaves only removed, (4) lateral leaves only removed, (5) every other primary and lateral leaf removed, and (6) all leaves removed in the fruit zone. The effect of north and south exposure of the vine in each treatment on C13-norisoprenoids was also evaluated. Wines were produced from these grapes according to standard red winemaking procedures. The C13-norisoprenoids were isolated by liquid/liquid extraction and simultaneous distillation and extraction. For quantitation, the GC-MSD was operated in selected ion monitoring mode. Peak areas for *m/z* 121, 157, and 192 were used for the measurement of β–damasceneone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and *trans*-vitispirane, respectively. Treatment 6 showed the largest amount of TDN and *trans*-vitispirane followed by treatment 5. In the more shaded treatments (1, 2, 3, 4), C13-norisoprenoid levels were variable. Formation of TDN

Abstracts for session presentations are in the approximate order as noted in the conference program. There are no abstracts included for the Soil Environment & Vine Mineral Nutrition Symposium, Sanitation Session, industrial posters, keynote speakers, and merit and honorary research presentations.
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and *trans*-vitispirane in grape and wine is generally affected by sunlight exposure; however, the relatively high amount of TDN and *trans*-vitispirane in control and ECTC samples might be due to the microclimatic variations independent of sunlight exposure. β–Damasceneone was not greatly affected by sunlight exposure. The grapes and wines from the south side of vines had higher contents of β–damasceneone, TDN, and *trans*-vitispirane than those from the north side of vines.

Wine Composition — Student

Binding Capacity of Insoluble Berry Cell Wall Material for Tannin and Large Polymeric Pigments

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Tannins and polymeric pigments are some of the most abundant and important phenolics found in grapes and wine. They are known to interact with proteins and polysaccharides, but their behavior with the insoluble cell wall matrix of grape berries has received little attention. We developed a convenient assay to measure the tannin-binding capacity of cell walls from berry skins and mesocarp tissue and used this assay to study the binding capacity of fruit during ripening. The ability of cell walls to bind tannins generally increased during ripening. We found that mesocarp cell walls had about twice the capacity to bind tannins compared with skin cell walls. We also examined the binding capacity in several varieties and across growing regions. We studied adjacent Cabernet Sauvignon vineyards in Napa Valley giving wines with drastically different levels of tannin in finished wine even though the fruit contained almost identical levels of tannin at harvest. Berry cell walls from a vineyard giving low tannin wines had a higher tannin-binding capacity per berry than another vineyard giving wines with higher tannin levels. This suggests that the tannin-binding capacity of cell walls may influence extractability of tannins from fruit during fermentation. Chemical fractionation of the insoluble cell wall matrix indicated that cellulose and hemicellulose play a role in tannin binding whereas the chelator-soluble pectins do not. This work suggests that the tannin-binding capacity of berry cell walls may be an unrecognized factor that can influence tannin extraction and retention in finished wines.

Prediction of Color Due to Copigmentation Components by Indirect Spectral Methods (NIR and MIR) and Partial Least Squares

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Two sets of young red wines were analyzed for free anthocyanins, copigmentation and polymeric pigment. One set was a series of 20 Californian wines that ranged from colorless to deeply colored (10 AU). The second set comprised 145 Italian wines of commercial origin, from many different wine regions and cultivars. The wines ranged in age from one to three years based on the vintage date. Spectra were obtained in two wavelength regions of the infrared: 600 cm-1 to 4000 cm-1 in the mid-infrared (MIR) and 900 μm to 2700 μm in the near-infrared (NIR). Partial least squares (PLS) regression was used to fit the spectral data to the chemical data. Initially all of the spectral data was used. This spectral data set was then separated into its NIR and MIR components. Finally, these were then further refined by selecting only wavelengths that made significant contributions to explaining the variance of the sample set. It was found that the NIR spectra were not able to give good fits to the analytical data; however, some of the color parameters were well correlated by the MIR spectra. The PLS model obtained from one set of wines did not predict the color parameters of the other set. This indicates that the PLS model is a result of over-fitting and that insufficient data is contained in the infrared region of the spectrum to predict these color parameters with confidence by a general model.

Pruning Effects on 2-Methoxy-3-Isobutylpyrazine Concentration in Cabernet Sauvignon Using a New SPME GC-MS Quantification Method

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2-Methoxy-3-isobutylpyrazine (MIBP) is an aroma compound that produces the vegetative aroma of bell peppers and also produces vegetative aromas in some wine varieties such as Cabernet Sauvignon, Merlot, and Sauvignon blanc. A solid-phase microextraction (SPME) stable isotope dilution GC-MS method for MIBP quantification in red wine was developed that required minimal sample preparation time. Wines with 30% (w/v) NaCl and 2-methoxy-$^2H_3$-3-isobutylmethoxypyrazine ([$^2H_3$] MIBP) internal standard were sampled using a 2-cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber for 30 min at 40ºC and analyzed by GC-MS in selective ion monitoring mode. The method showed good linearity from 1 to 100 parts per trillion and a limit of detection of 2 ng/L in wine. MIBP concentrations in Cabernet Sauvignon wines that were produced from six winter pruning treatments over two vintages were analyzed using the method. The number of buds per vine was significantly negatively correlated with the wine MIBP concentrations ($p < 0.001$). In addition, MIBP concentration was directly correlated to sensory vegetal intensity ratings obtained by descriptive analysis ($p < 0.05$).

Influences of Viticultural Practices on the Aroma and Tannin Profile of Napa Valley Cabernet Sauvignon Wines

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To reveal the relationship between simultaneous multiple viticultural practices and the sensory and chemical characteristics of final wines, 12 viticultural practices—vineyard block, rootstock, vineyard location, vine density, shoot number, trellis, overall water, orientation, vine age, fertilization (nitrogen

and potassium), and pruning method—were varied using existing Cabernet Sauvignon viticultural trials at the Oakville Experimental Vineyards over three vintages from 2000 to 2002. In all, 38 combinations of treatments were chosen each year. The grapes from each lot were harvested at 24.0 Brix (±0.4 Brix) and identically processed into the final wines in the UC Davis research winery. Chemical analysis was completed after approximately four months of aging. After 10 months of aging, a trained panel of judges rated the intensity of aroma attributes (cocoa, cherry, berry, dried fruit, vanilla, green pepper, cooked veggie, olive, pepper, and mushroom) and three taste attributes (bitterness, sourness, and astringency). Data were analyzed by multivariate statistical analysis to examine differences among wines and the three vintages. General regression neural networks and other database mining methods were used to identify the viticultural practices that have the largest influence on chemical and sensory properties of the wines. Models were formed to predict the effects of these critical practices on final wine qualities. This type of model can be used by vineyard managers to achieve specific wine characteristics for whole vineyards or even small groups of vines, leading to the ability to perform precision viticulture in the future.

Spatial Variability of Grape Phenolics: Using Precision Viticulture Tools to Optimize Wine Phenolic Composition

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This research investigated how spatial variability due to soil/landscape characteristics within a commercial vineyard influences vine growth and ultimately wine chemistry (emphasis on phenolics) and determined the technical feasibility of using precision viticulture tools to manage wine style in cool-climate vineyards. The study was conducted in two commercial vineyards where blocks within each vineyard consist of similar plant material and vineyard management practices. Monitored vineyard parameters collected by GPS location include soil characteristics, water relations, vine growth, and crop load. Geospatial maps of vine vigor were used to segregate vines (within each vineyard block) into high, medium, and low vigor wine lots. Fruit samples were collected from these wine lots at veraison and harvest. Research wines were produced from each wine lot. Fruit parameters included cluster and berry weights, seed color, number and weight, and complete grape composition (pH, TA, sugar, phenolics, and nutrient status). Standard wine measurements were performed as well as in-depth HPLC analysis of low molecular weight phenolics and tannins (degree of polymerization, subunit composition, skin versus seed). Vineyard data indicate that significant differences in vine vigor and fruitfulness exist within each block. Differences in fruit composition (pH, TA, and Brix) were found at veraison and harvest for wine lots. Data on wines show clear differences in yeast assimilable nitrogen content, amino acids, color density, phenolics, and fermentation rates, all of which can be tied to differences in vine vigor.

Vine and Berry Physiology — Student

Seasonal Patterns of Root Physiology and Dynamics of *Vitis vinifera* cv. Merlot on Two Rootstocks under Different Levels of Irrigation

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Previous determination of the effects of wet and dry soil conditions on spatial and temporal vine water status has been largely restricted to the aboveground portions of the plant. We examined root dynamics within a single vine in wet and dry soil using minirhizotron technology. We compared the effects of three irrigation treatments (0%, 40%, 100% of ET_c) on two rootstocks that differ in overall vigor (1103P and 101-14 Mgt) in a Merlot block in Oakville, CA. Irrigation treatments maintained stem water potentials between –1.0 and –1.2 MPa. To better understand both spatial and seasonal root physiology and dynamics, roots were continually monitored using a specially designed camera that could be inserted into clear minirhizotron tubes in the vineyard. The majority of new roots were produced between 30 and 90 cm from the soil surface on the irrigated side of the vine. Results in the second year indicated significant differences between the performance of 1103P and 101-14 Mgt rootstocks in response to irrigation deprivation. Without irrigation, 1103P had greater root survivorship than 101-14 Mgt. Root survivorship declined under deficit irrigation for both rootstocks. Root lifespan strongly diminished with phylloxera infection regardless of irrigation level or rootstock. A root that became infected with phylloxera had a median lifespan of 35 to 100 days compared to several hundred days for noninfected roots. Our current results indicate a strong difference in root production and survivorship between individual rootstocks in response to varying levels of irrigation.

Berry Turgor Pressure: Direct Measurement in *Vitis vinifera* throughout Development and Relationship to Vine Water Status and Deformability

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Veraison marks the onset of particular physiological changes, including resumption of growth, adjustments in the berry water budget, increased deformability, and the onset of sugar accumulation. The pressure microprobe was used to determine berry turgor pressure directly and in situ. Berry turgor pressure was not significantly different at increasing depths from the berry surface. Berry turgor pressure increased as growth slowed in Stage II, then decreased prior to veraison. Analysis of Chardonnay, Cabernet Sauvignon, and Pinot noir revealed that the decline in turgor pressure precedes the significant increase in softening and sugar accumulation observed at veraison, but turgor pressure remains relatively constant and low (<0.5 Bars) after the onset of ripening. Berry turgor pressure was up to 10 times higher before veraison than after veraison. When irrigation was withheld, turgor pressure decreased with vine water

status before veraison, but was insensitive to similar decreases in vine water status after veraison. Results indicate that veraison involves loss of turgor pressure and is consistent with the hypothesis that the postveraison berry is hydraulically isolated from the vine.

Using Whole-Vine Photosynthesis to Understand the Effects of Water Stress on Premium Winegrapes

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A six-chamber, mobile field laboratory was used to measure whole-vine photosynthesis from field-grown, own-rooted, drip-irrigated *Vitis vinifera* cv. Cabernet Sauvignon under three regimes of regulated deficit irrigation (RDI): (1) standard RDI (70% of vine evapotranspiration, ET, was replaced weekly); (2) early deficit (50% of vine ET was replaced weekly between fruit set and veraison); and (3) veraison deficit (50% of vine ET was replaced weekly between veraison and harvest). When not under 50% deficit, vines in regimes 2 and 3 were irrigated according to standard RDI practice. After harvest all vines were well irrigated until leaf fall. Vines were planted in 1992 in rows oriented N-S, with spacing of 6 feet between vines and 9 feet between rows at Paterson, WA. Whole-vine chambers were deployed for 7-day measurement runs during physiologically important stages: fruit set, pre- and postveraison, and pre- and postharvest. Chambers measured simultaneously two vines per treatment, data collected during 48 hr, then the chambers moved to nearby vines until six vines per treatment were sampled. On adjacent vines, single-leaf measurements of photosynthesis were collected at the same time as the whole-vine measurements. Leaf area per vine was estimated at each stage. Large differences were observed in net carbon exchange and in transpiration between vines under the standard RDI practice and those under the additional water stress. In the preveraison period, early deficit vines fixed up to 40% less carbon during the middle of the day than did vines under standard RDI. A similar reduction was observed in sunlit, single leaves measured independently of the whole-vine chambers. Vines under early deficit transpired up to 62% less than those under standard RDI. Only small differences were detected in net carbon exchange and transpiration before and after harvest, when daytime temperatures were lower and day length was shorter.

Effect of Viticultural Practices on Biotin, Pantothenic Acid, and Assimilable Nitrogen Concentrations in Grape Must

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Grapes were sampled from nonexperimental and two experimental vineyard blocks from 2001 to 2003 and analyzed for biotin, pantothenic acid, and nitrogen. While vitamin analyses relied on microbiological assays, assimilable nitrogens were measured using NOPA and a selective ion electrode for ammonia. In nonexperimental vineyards, significant differences ($p < 0.05$) were demonstrated for all three nutrients between year, cultivar, vineyard, and region. The first experimental vineyard used supplementary water application in addition to standard irrigation practices in different areas of the canopy. Although year was a significant factor ($p < 0.05$) for all nutrients, only biotin and assimilable nitrogen exhibited statistical differences ($p < 0.05$) between treatments. The second experimental vineyard employed a 3 X 2 factorial experimental design with three irrigation regimes and two crop loads. Year and treatment factors were significant for all three nutrients ($p < 0.05$), but only pantothenic acid and nitrogen showed year X treatment interactions. Differences in the deficit irrigation treatments for pantothenic acid were seen only in the lower cropping level compared to the higher level. While most samples analyzed contained adequate amounts of biotin and pantothenic acid, exceptions were noted, especially in 2003 when concentrations of biotin and assimilable nitrogen were quite low. Viticultural practices appear to have some effects on the biotin and pantothenic acid content, but obvious trends were difficult to establish.

Wine Fermentation — Student

Fermentation of High-Density Chardonnay and Cabernet Sauvignon by Strains of *Saccharomyces cerevisiae*

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Extended maturation of winegrapes for flavor development is currently receiving much attention from the wine industry. The resulting long hang time leads to increased must density. The aim of this study was to evaluate the performance of commercially available wine yeast strains in high-density must fermentations. Cabernet Sauvignon and Chardonnay musts averaging 30.1 and 27.9 Brix, respectively, were processed as small-scale fermentations in triplicate using commercially available strains of *Saccharomyces cerevisiae*. Five yeast strains were selected for Cabernet Sauvignon and five for Chardonnay. Selection was based upon the manufacturer's recommendation for potential alcohol tolerance, fermentation temperature preferences, and varietal suitability. Flow cytometry was used to evaluate, enumerate, and determine the viability of inoculated yeasts throughout the fermentation. FTIR spectroscopy was used to measure chemical changes. Sensory analyses will be performed after three months of bottle aging. Flow cytometer data showed differences in lag times as well as kinetics of growth and death rates. FTIR revealed differences in fermentation proficiency as well as production of volatile acidity, glycerol, and Folin-Ciocalteu index. Four out of five yeasts fermented the Cabernet Sauvignon to dryness. Three of the five yeasts fermented the Chardonnay completely.

Juice Composition: Its Role in Problem Fermentations

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Sluggish and stuck fermentations are a perplexing and chronic wine industry problem. The ability to predict fermentation outcome prior to the beginning of fermentation would allow winemakers to take corrective action while such measures are still effective. Thus, the objective of this two-year research study was to ascertain the impact of various juice components on fermentation kinetics. Compositional analysis was performed on Chardonnay juice samples provided by commercial wineries throughout California from the harvests of 2001 and 2002. The samples were divided into four categories based on their fermentation profiles: fast, medium, sluggish, and stuck. All samples were analyzed for organic acids, glucose, fructose, ammonia, α-amino nitrogen, amino acids, vitamins, and metal ions. Total bacterial concentration and *Hanseniaspora* concentration were also measured by quantitative PCR. The data were then analyzed using decision tree analysis and partial least squares to determine which components were most significant in predicting problem fermentations. Data from year one of the study (202 samples) and year two (323 samples) were analyzed separately and combined. Year one of the study determined that low fermentation temperatures, high concentration of glucose and nicotinic acid, and low concentrations of malic acid, serine, glutamate, magnesium, and asparagine were most significant in predicting poor fermentation behavior. Factors identified in the second year of the trial, as well as in the combined data set, will be presented, together with methods for utilizing these findings to predict fermentation kinetics based solely on juice characteristics and the intended processing.

Inhibition of Alcoholic Fermentation by Bacteria Isolated from Stuck Fermentations

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Bacteria found in wines undergoing sluggish or stuck alcoholic fermentations were isolated and characterized. Thirty-five morphologically different strains were isolated from samples obtained from wineries in California, Washington, and other locations. These bacteria were screened for antagonism against *Saccharomyces cerevisiae* UCD 522 using an agar overlay assay. Fermentations of Chardonnay and Merlot grape juices containing both yeast and bacteria were also conducted. Of the 35 isolates, 18 exhibited strong inhibition of UCD 522 evidenced by clearing zones. While most of the recently isolated strains have been confirmed as being *Lactobacillus hilgardii* by 16S rRNA sequencing, strains of *Oenococcus oeni* and *Acetobacter* were also identified. Two previously isolated strains of *L. hilgardii*, M1a and S1F8, inhibited fermentation of the Chardonnay juice induced by *Saccharomyces bayanus*. While Chardonnay juices inoculated with only *S. bayanus* went dry, the presence of either of these bacterial strains yielded stuck fermentations (6.0% and 7.4% v/v alcohol), lower pH (3.32 and 3.42), and higher volatile acidity (0.40 and 0.26 g/mL) as compared with the control wine (11.9% v/v alcohol, pH 3.78, and 0.023 g/mL VA). Resistance of these spoilage bacteria to SO_2 or lysozyme has not been observed. These results indicate that *Lactobacillus hilgardii* can be a cause of stuck or sluggish alcoholic fermentations.

Inhibition of Malolactic Fermentation by Wine Yeast during Alcoholic Fermentation

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This study investigated the complex metabolic interactions that occur between wine yeast (*Saccharomyces*) and malolactic bacteria (*Oenococcus*). Alcoholic fermentations were induced in a synthetic grape juice using different yeast strains, and samples were periodically removed, sterile filtered, and inoculated with *O. oeni* to induce malolactic fermentation. Prior to inoculation with *O. oeni*, some samples were treated with protease or a concentrated nutrient solution. During alcoholic fermentation, *S. cerevisiae* strain V1116 produced the highest levels of SO_2 and these media were highly inhibitory to malolactic fermentation. Neither the addition of nutrients nor protease relieved this bacterial inhibition. MLF was highly inhibited in media fermented by *S. cerevisiae* strain RubyFERM but not strain EC1118 despite production of similar amounts of total SO_2. The addition of nutrients to RubyFERM-fermented media did not decrease bacterial inhibition; however, bacterial inhibition was relieved after addition of protease. These findings suggest that the inhibition of malolactic fermentation by *S. cerevisiae* is caused by a number of mechanisms including the synthesis of SO_2 and/or antibacterial peptides/proteins.

Effect of Yeast Selection on the Phenolic Profile of Red Wines

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Phenolic compounds are important to red wine quality because they impact color, mouthfeel, and ageability. Previous work has shown that winemaking practices can have a greater effect on the tannin profile of the finished wine than variety, vintage, and vineyard location. In order to determine if yeast selection is one of these winemaking practices, Pinot noir, Cabernet Sauvignon, and Syrah musts were fermented with different yeast strains. The phenolic profile of the resulting wines were analyzed. For each must, small lots were fermented in a temperature-controlled incubator with five *Saccharomyces* strains in triplicate. Sterilized equipment and a high inoculum of the desired yeast strains were used. The yeast population of the fermentations was monitored by tip-plating on WL media, which indicated that *Saccharomyces* was the dominant yeast, with little or no wild yeast present. The amount of anthocyanin, small polymeric pigment, large polymeric pigment, tannin, and total phenols in the resulting wines was measured using a protein precipitation assay.

The must used had a greater effect on fermentation rate than yeast strain, with fermentation rates decreasing in the following order: Pinot noir>Cabernet Sauvignon>Syrah. The Cabernet Sauvignon and Syrah phenolics data were analyzed using ANOVA. There was no significant difference ($p > 0.05$) among yeast replications for all of the phenolic parameters except small polymeric pigment. There were significant differences between yeast strains for anthocyanin, large polymeric pigment, tannin, and total phenols. The stirring rate of the fermentations dramatically affected the tannin content of the finished wines.

Biochemistry/Genetics — Student

Abscisic Acid: A New Tool for Improving the Color of Table Grapes

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Inadequate color development is a major problem in many California table-grape vineyards, reducing fruit quality and packable yields. The only chemical tool presently available for improving berry color is the plant growth regulator ethephon (2-chloroethylphosphonic acid). When applied near veraison, ethephon stimulates both color and sugar accumulation in table grapes. However, the efficacy of ethephon on color development is often erratic, and its application may result in detectible berry softening at harvest. Abscisic acid (ABA) is an important plant hormone involved in the regulation of many physiological processes, including stomatal opening and seed dormancy. Previous investigations also indicated that ABA stimulated grape color development. Experiments conducted in 2003 examined the effects of ABA on the color development of table grapes in the San Joaquin Valley, California's primary table-grape producing region. Mature Flame Seedless, Redglobe, and Crimson Seedless grapevines were treated with 250, 500, or 1,000 mg/L ABA. A group of untreated clusters, as well as clusters treated with ethephon (250 mg/L; the standard industry treatment), were also included in the experiments. ABA dramatically increased the pigment content of all cultivars tested. The pigment contents of ABA-treated (500 mg/L) Flame Seedless and Crimson Seedless berries were 5-fold and 30-fold greater, respectively, compared with their controls. However, ABA concentrations ≥500 mg/L resulted in fruit which appeared black in color, instead of the desired red color characteristic of each cultivar, and also decreased berry firmness. In contrast, ethephon provided only minor increases in berry color compared to the control.

Determining the Identity of Valtellina Grape Varieties

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The grapes of Valtellina and Friuli are poorly known in California. Even within the region there is confusion regarding clones and types of a given variety. To clarify the true identity of these regional varieties, DNA from 28 of the winegrape varieties/clones from this region were analyzed. The hypothesis being tested was that there were no DNA differences among these varieties. SSR markers from eight loci were used to distinguish the grape samples. Five Nebbiolo clones were examined and genetic differences were detected. A sixth Nebbiolo clone was identified as Rossolino Nero #40. Two Friulian varieties were examined; both were labeled Pignolo and were found to be genetically different. One of these varieties was an exact match to the Valtellina Pignola variety examined. From this testing, 11 new varieties were added to the UC Davis Grape DNA database. Additionally, the DNA profiles of the following six varieties did not match the DNA profile of that named variety in the UC Davis database (database name in parenthesis): Cabernet Franc (Rabusa), Corvino (Rossoletta), Merlina (Teroldico, Teroldego 1), Rossola 29 (Rossolino nero 40), Rossola 40 (Rossolino nero 40), and Rossola Verde (Rossola 2).

Development of a Genetic Map for the Interspecific Rootstock Cross Ramsey x Riparia Gloire

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The F1 population of the interspecific rootstock cross Ramsey (*Vitis champinii*) x Riparia Gloire (*Vitis riparia*) is expected to segregate for many important rootstock traits based on the diverse nature of its parents. Ramsey is a nematode-resistant, high-vigor rootstock, with expected drought, lime tolerance. Riparia Gloire, however, is known as a shallow-rooted, low-vigor stock, and is susceptible to nematodes. We are currently creating a linkage map of an F1 population derived from these rootstock parents, using microsatellite and AFLP markers. Markers are visualized with silver staining, and segregation data is analyzed using both JoinMap and Mapmaker software. The purpose of this framework map is to provide a basis for mapping root-knot nematode (*Meloidogyne incognita*) resistance, rooting angle, and lime tolerance. The mapping population consists of 186 individuals, and approximately 100 markers have been tested on this population to date. The goal of the framework map is to place evenly spaced markers on each of 19 linkage groups, corresponding to the 19 *Vitis* chromosomes. The first trait to be mapped will be root-knot nematode resistance. Preliminary screening data places this dominant trait with microsatellite markers on chromosome.

Screening for *Vitis arizonica*-Derived *Xiphinema index* Resistance

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The dagger nematode, *Xiphinema index*, is one of the most damaging grape-root pests, and few resistant rootstocks are available. It has been documented that *Vitis arizonica* has strong resistance to *X. index*, and initial screens suggest that resistance is due to a single locus. To identify the inheritance of *X. index* resistance and to place the trait on a genetic linkage map, a greenhouse screen was conducted on the 9621 popula-

tion. The 9621 population consists of 181 individuals and was derived from a cross of *X. index* resistant D8909-15 x susceptible F8909-17. The D8909-15 is a cross of *Vitis rupestris* x *V. arizonica* and F8909-17 is a cross of *V. rupestris* x *V. arizonica/candicans.* Green cuttings were collected from mature field-grown vines of the 9621 population. They were rooted in sponges and four replicates of each were planted in 5-cm^3 pots. The potting mixture was composed of 75% course sand and 25% sterilized soil, and the pots were placed on capillary mats. Nematodes were collected from infested soils using the Baermann funnel technique. One hundred *X. index* were placed in each pot and symptoms were allowed to develop for 6 to 10 weeks, after which the roots were examined for gall formation. Results indicate that resistance is controlled by a single dominant gene segregating 1:1 in the population. Preliminary mapping efforts with this resistance trait place it on linkage group 19.

Resistance to Pierce's Disease in *Muscadinia rotundifolia* and Other Native Grape Species

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Pierce's disease (PD), caused by the bacterium *Xylella fastidiosa*, is an important disease of grapevines. Grape species native to areas of the United States where the disease is severe appear to have evolved resistance to PD. In this study, we make a quantitative assessment of the level of resistance in cultivated and wild selections of five native grape species and one complex hybrid. Using controlled greenhouse trials, our conclusions are based on estimates of bacterial concentrations in stem tissue via ELISA and subjective evaluations of disease symptoms. *Vitis labrusca*, native to the northeast United States where PD is absent, appears to be as susceptible as *Vitis vinifera*. California natives *Vitis californica* and *Vitis girdiana* appear to be moderately susceptible, although there was significant variation among the *V. girdiana* selections. In contrast, *Muscadinia rotundifolia* and *Vitis arizonica*, both native to areas of severe disease pressure, appear to be very resistant. We also sought to test if this pattern of resistance tracking disease pressure was evident within species. Indeed, wild accessions of *M. rotundifolia* from areas where PD is rare supported up to a 20x higher concentration of bacteria than the accessions from areas where PD is severe. Trials with wild accessions of *V. girdiana* showed a similar pattern, with susceptible selections supporting up to 100x higher bacterial concentrations. These results, detailing a gradient in resistance both among and within species, are consistent with the hypothesis that PD resistance has evolved in response to disease pressure. This study identifies prime plant material for breeding projects as well as research efforts aimed at uncovering the genetic and physiological details of PD resistance in native grapes.

Analysis of Phenolic Glucosyltransferases in Grape Cells

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As a last step in their synthesis, many phenolic compounds in grapes are glucosylated. This step increases their water solubility and, in the case of anthocyanins, stabilizes the molecule and prevents its breakdown. The enzyme responsible for the glucosylation of flavonoids in grape is UDP-glucose: flavonoid glucosyltransferase (UFGT). Southern blot analysis of the *Vitis vinifera* genome shows shows a single band when probed for UFGT. Current genomic efforts in *Vitis* have elucidated two different gene sequences (UFGT1 and UFGT2). We analyzed the activity of phenolic glucosyltransferases of grape cell cultures (cv. Gamay Freaux) grown under three light conditions: dark, constant light, and light supplemented with a 10-min irradiation with UV-C light. Based on chemical production data and enzyme activity assays, there appear to be three distinct phenolic glucosylation activities in enzyme extracts. The glucosylation of anthocyanidins, forming the more stable anthocyanins, is stimulated by growth of the cells in the light when compared to growth in the darkness. Another glucosyltransferase activity, this one specific for flavonols, is induced by UV irradiation of the cells. Lastly, glucosylation of a stilbene (resveratrol) is not light responsive, with about equal activity under the conditions tested.

Precision Viticulture

Use of a Geographic Information System to Study Mesoclimate Variation in Okanagan Valley Vineyards

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A geographic information system (GIS) has been constructed to study relationships among site conditions, management practices, and winegrape quality in the Okanagan Valley in British Columbia. Most vineyards in the Valley are sited on, or surrounded by, rough terrain, which is suspected to cause variable mesoclimates that affect vine development and fruit maturation. A study was conducted to characterize mesoclimate variation and determine the relative effects of insolation and elevation on temperature variation. Temperature was monitored in two vineyards using networks of temperature sensors. Insolation was calculated as a function of slope, aspect, and solar angle. Variation in temperature within the vineyards was as high as 8ºC and was unrelated to insolation. Temperature appeared to be affected most by air movement influenced by terrain within and surrounding the vineyards. Basic composition of fruit from the sensor sites was examined to determine the degree to which fruit maturation was affected by temperature and insolation. Initial results indicate that insolation is less important than temperature in affecting fruit development.

Influence of Drip Irrigation Flow Rates on Spatial Variability of Vine Growth

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We examined the hypothesis that irrigation history might influence spatial variability of vine growth in a hillside Pinot noir vineyard fitted with a drip irrigation system in the Carneros district of Napa Valley, California. The vineyard was planted on 3.22 acres of Haire Clay Loam soils. Drip-emitter flow rates in the vineyard were calculated using the volume of water (V) dispensed by each emitter for a time (t) of 20 seconds (flow rate = V/t). The water volume delivered was recorded and displayed on a map of the vineyard, using GIS software. The emitters were located at the foot of previously geo-referenced data vines, recorded using a Trimble GPS with an accuracy of about 1 meter. Substantial spatial variability was observed for a number of plant and fruit characteristics, including yield, fruit ripeness, pruning weights, and plant size. Pressure-compensated drip irrigation lines, with standard flow rates of 2 L/hr per emitter are used in the vineyard, but rarely delivered uniform water amounts at different emitters for different technical reasons. The average rates measured for emitters ranged from 0.9 L/hr to 3.5 L/hr (mean = 2.23, SE = 0.31). Although it was hypothesized that the strong observed variability in plant performance may be attributed, in part, to differences in drip emission rates within the vineyard, correlations between the drip emitter rates and vine variability did not bear this out. It is thus concluded that other site characteristics, most likely spatial variation in soil properties, are driving historical differences in vine growth.

Developing Site-Specific Irrigation Management Strategies for a Cabernet Sauvignon Vineyard

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Precision farming consists of a group of technologies that allow the management of spatial variability of the microclimate, soil characteristics, and vineyard vigor. Implementing precision farming principles could permit the site-specific management of irrigation in the vineyard to improve must and wine quality. A study was carried out during the 2001 to 2002 and 2002 to 2003 growing seasons on a commercial Cabernet Sauvignon vineyard located in the Pencahue Valley, Maule region of Chile (lat. 35°22'S; long. 71°47'W). The study included the use of aerial imagery, automatic weather stations, water potential assessments, soil moisture instruments, global positioning system, and a geographic information system. The effect of different irrigation levels on yield and grape maturity varied depending on the vigor levels of the vines growing at different locations within the vineyard. The results of this study indicated that the development of maps with homogeneous sectors (according to the spatial variability of the soil, water consumption, and vineyard vigor) could be used to reduce the water applications to the vineyard and increase the must quality. However, to improve must quality using regulated deficit irrigation, a site-specific calibration of stress coefficients is required for each homogeneous sector within the vineyard.

Brettanomyces Seminar

Genetic Diversity among *Brettanomyces*

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Brettanomyces, the nonsporulating form of the genus *Dekkera*, includes four species (*B. anomalus, B. bruxellensis, B. naardensis,* and *B. custerianus*). The fifth species *B. nanus* was added after renaming *Eiella nana* based on the rDNA sequence homology. So far all strains of *Brettanomyces* isolated from wine belong to the species *B. bruxellensis*. Wines affected with *B. bruxellensis* are described as having a range of odors, such as smoky, spicy, burnt plastic, Band-Aid, barnyard, sweaty horse, leather, wet dog, and mousy and bitter aftertastes. Electrophoretic karyotyping, restriction fragment length polymorphism analysis (RFLP), and random amplified polymorphic DNA (RAPD)-PCR were compared for a set of native wine isolates. Karyotyping revealed significant variation among the chromosomal patterns of *Brettanomyces/Dekkera* strains, but did not allow grouping of isolates into species. RFLP did not offer any significant advantage over karyotyping. RAPD-PCR fingerprints varied for all *Dekkera* species and intraspecific differences were observed only when applied in combination with other fingerprint techniques. We investigated the genetic relationship among *B. bruxellensis* strains by sequencing portions of the 26S rDNA region of the actin gene. The polymorphism in the sequence of the rDNA fragment allowed us to compile 48 strains of *B. bruxellensis* into six groups. Higher polymorphism was observed in the actin gene fragment. The country of origin is not correlated to the genetic differences detected. Further studies will compare physiological and flavor characteristic differences with genetic profiles. Genetic fingerprints already allow the tracking of *Brettanomyces* within the winery environment from various sources.

Physiological Diversity of *Brettanomyces/Dekkera* Isolated from Wine

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Brettanomyces/Dekkera isolates from the wine yeast culture collections at UC Davis and Cornell University in Geneva, New York were examined for physiological and metabolic diversity under laboratory conditions. Thirty-five isolates were chosen for analysis based upon the type of wine, vintage, and geographic area where they were isolated. Strains were tested for growth on various carbon and nitrogen sources, vitamins, and under different environmental conditions. The level of production of 4-ethylphenol and 4-ethylguaiacol in a wine made from Grenache grapes was determined. The isolates have an absolute requirement for the vitamins biotin and thiamin but do not require any amino acids for growth. Most or all of the isolates grew well on cellobiose, galactose, maltose, sucrose, and trehalose. All strains grew on arginine as a carbon and nitrogen source. *Brettanomyces* strains differed widely in their production of 4-

ethylphenol and 4-ethylguaiacol and their tolerance to both high and low temperature and sulfite when grown in minimal media. Most isolates grew well at pH 2 and up to 10% ethanol. Using a classic taxonomic scheme for identifying *Brettanomyces/Dekkera* (Kurtzman and Fell 1998), 14 isolates keyed out as *Dekkera anomala*, one was not able to be classified, and 20 were *Dekkera bruxellensis*. A preliminary taxonomic study based on PCR and sequencing of the D1-D2 region of the 26s rDNA of each isolate showed that 34 were *Dekkera bruxellensis* and one was *Dekkera anomala*.

Dissemination of 4-Ethylphenol Producing Yeast Species from the Vineyard to the Bottle

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The ability to produce 4-ethylphenol is an unwanted feature of yeasts species associated with wine production. The species *Brettanomyces bruxellensis*, or its teleomorph *Dekkera bruxellensis*, is presently regarded as the sole yeast producing 4-ethylphenol in amounts that significantly affect wine aroma and flavor. However, ecological studies of wine-related environments have not to date specifically addressed isolation and identification of other yeasts sharing this ability. Thus, the aim of this work was to evaluate the dissemination of 4-ethylphenol producing yeasts throughout the wine production process, from grapes and insects in the vineyard to bottled products. Sample analysis was based on the utilization of a selective and differential medium (DBDM) aimed at the recovery of *D. bruxellensis*, either by plating or by the most probable number technique. This technique was essential for accurate estimation of numbers of colony forming units of *D. bruxellensis* in mold-contaminated samples and in samples highly contaminated by other microorganisms. The utilization of DBDM revealed the presence other species capable of 4-ethylphenol production, among which *P. guilliermondii* showed production efficiencies close to that of *D. bruxellensis*.

Novel Methods to Detect *Brettanomyces* (*Dekkera*) in Wine

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Traditional methods to detect the spoilage yeast *Brettanomyces (Dekkera) bruxellensis* in wine involve lengthy enrichments and often overlook metabolically active but nonculturable (ABNC) populations, which are of interest since they likely impact fermentation outcome and product quality. In order to enumerate the total *Brettanomyces* population, a number of direct detection methods have been developed, all of which avoid time-consuming enrichment steps. Some methods, such as flow cytometry or microscopy, use antibodies or nucleic acid based probes (respectively) to directly quantify yeasts in wine. Another strategy for direct detection is to use nucleic acid, isolated directly from wine, as a template upon which to survey microbial community structure and to quantify individual populations. We have developed a quantitative real-time PCR (QPCR) method to directly enumerate *B. bruxellensis* in wine. Specific PCR primers to *B. bruxellensis* were designed to the 26S ribosomal RNA gene that did not amplify other non-target yeast and bacteria common to the winery environment. The QPCR assay was linear over a range of cell concentrations (6 log units) and could detect as little as one cell per mL in wine. Addition of a large amount of nontarget yeasts did not impact the efficiency of the assay. This method will help identify various routes of *D. bruxellensis* infection in winery environments and aid studies on the relationship between total *Brettanomyces* population size and 4-ethylphenol production. Moreover, the less time-consuming assay (~3 hr) may allow winemakers to make wine-processing decisions more quickly, thus reducing the threat of spoilage by *B. bruxellensis*.

Behavior of *Dekkera bruxellensis* and *Pichia guilliermondii* in Wines

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The yeast species *Dekkera bruxellensis* and *Pichia guilliermondii* have the ability to produce high levels of 4-ethylphenol in synthetic media. However, the behavior of the latter species in wines is not known and the inactivation of *D. bruxellensis* in wines is not fully characterized. Our goal was to assess the behavior of these two species after inoculation in grape juices and in wines, under different levels of preservatives. The typical behavior of *D. bruxellensis* after inoculation in wines was described by an initial exponential death phase followed by growth up to about 10^8 UFC/mL and subsequent death. However, viability measurements using methylene blue evidenced the presence of a viable but nonculturable population. Sorbic acid was not an effective preservative, while the effectiveness of potassium metabisulfite and DMDC was dependent on ethanol content and pH value. *Pichia guilliermondii* was inhibited in wines without addition of preservatives.

Descriptive Analysis of *Brettanomyces*-Infected Cabernet Sauvignon Wines

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This study is a preliminary indication that it is possible to discriminate *Brettanomyces*-fermented wines based on the odors produced. Thirty-six lots of Cabernet Sauvignon wine were inoculated with 35 individual strains of *Brettanomyces* spp. (plus one control lot). Implantation of the inocula was determined by plating. After 46 days of incubation, the wines were sterile filtered and bottled. A descriptive panel (14 panelists) was assembled and trained to assess each wine in triplicate using seven descriptive terms: Band-Aid, soy, horsey, earthy, leather, tobacco, and putrid. The terms were developed using an adapted consensus method. Judges were trained with aroma

standards for the terms prepared in a neutral red wine. Wines were evaluated under controlled conditions, and data were collected using Fizz Acquisition Software. A general linear model analysis of variance for each of the descriptive terms shows that for five of the seven terms (Band-Aid, soy, horsey, earthy, and putrid), the variance in the data can be explained by the wines ($p < 0.06$). A principal component analysis (PCA) of the mean data indicated that the first two principal components (PC) explained 69% of the variance in the data set. The first PC discriminated wines that had *Brettanomyces* fermentations that did not add horsey, soy, Band-Aid, earthy, or putrid odors. Since the fermentations only lasted 46 days, it is possible that these strains of *Brettanomyces* were slow growing and had not yet started producing the odorants responsible for these odors. The second PC discriminated wines that had more Band-Aid and less earthy or putrid odors.

Incidence and Control of *Brettanomyces*: The Australian Perspective

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The incidence of *Dekkera/Brettanomyces* yeast-derived compounds in Australian red wines has been studied by surveying 303 Cabernet Sauvignon and Cabernet Sauvignon-Merlot wines from the vintages 1996 to 2002 for 4-ethylphenol (4EP) and 4-ethylguaiacol (4EG), and to a lesser extent, isovaleric acid. Mean 4EP concentrations for the vintages 1996 to 2000 were not significantly different (range 847 to 1164 ug/L); however, concentrations decreased for 2001 and 2002 wines (490 ug/L). Concentrations of 4EP and 4EG were correlated, but neither 4EP nor 4EG concentrations were correlated with isovaleric acid. 4EP:4EG ranged from 2 to 38 and there appeared to be differences in this ratio between some regions of grape production. Chemical and sensory analysis of 72 wines showed that 4EP concentration correlated with the mean scores of the sensory panel for the attributes overall *Brettanomyces* aroma, Band-Aid/medicinal aroma, and metallic taste, but neither 4EP nor isovaleric acid concentration correlated with sweaty/cheesy aroma. 58 of 65 isolates from 20 wineries were confirmed as *Dekkera bruxellensis* by ITS-PCR-RFLP. Amplified fragment length polymorphic (AFLP) analysis of 41 isolates suggested a prevalent dominant cluster/strain present across most regions studied; however, extensive genetic diversity existed among strains for several wineries. Control measures for *Dekkera/Brettanomyces* advocated by the AWRI include general hygiene, management of residual nutrient concentrations, optimization of the effectiveness of SO_2 additions, pH management, lowering of wine turbidity/improvement in wine clarification procedures, and barrel management and may account for the observed decrease in 4EP content in 2001 and 2002. The 303 bottled wines surveyed showed an inverse correlation between 4EP and residual sugar content, possibly due to the sugar being used as a substrate for cell growth, which may be related to 4EP production.

Effect of Different Barrique Sanitation Procedures on Yeasts Isolated from the Inner Layers of Wood

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In wines the risk of microbial spoilage is particularly relevant in wooden barrels used for wine aging, mainly because it is difficult to sanitize the wood properly. This work was aimed at the evaluation of the effect of several sanitation procedures on the reduction of total microbial flora and of *Dekkera bruxellensis* counts. A group of used oak barrels contaminated with *D. bruxellensis* were differently treated and were dismantled to analyze samples of wood shaves taken from internal surfaces at different depths. Absence of contamination was only observed with steam treatment and in the upper level (0 to 2 mm) of the staves. With this treatment complete destruction of the contaminating flora was not achieved in any level of stave side surfaces and in grooves. The presence of *D. bruxellensis* was detected in depths up to 8 mm in the wood corresponding to the level of wine penetration.

Identification and Analysis of 4-Ethyl Catechol in Wines Tainted by *Brettanomyces* Off-Flavor

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For the first time, the compound 4-ethyl catechol (4-ethyl-benzene-1,2-diol) is reported in wines affected by an off-flavor associated with the yeast *Dekkera (Brettanomyces) bruxellensis*. This compound presumably arises from caffeic acid as precursor, in analogous fashion to the previously reported *Dekkera*-associated compounds 4-ethylphenol and 4-ethylguaiacol arising from *p*-coumaric and *p*-ferulic acids, respectively. 4-Ethyl catechol could be detected in wine aroma extracts only after derivatization, which explains why it has not previously been reported. A GC-MS method for detection and quantification of all three ethyl compounds and their determination is described. The mass spectra of the derivatized compounds and internal standard are reported. Data on the levels of 4-ethyl catechol to be found in wines of different origins are given. The amount of 4-ethyl catechol found with respect to levels of the other two 4-ethylphenols seems to be dependent on grape variety. The odor of 4-ethyl catechol can be described as horsey.

Unraveling the Mystery of *Brettanomyces* Flavor

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Beer and wine fermentations often contain *Brettanomyces*, a yeast considered to be both a spoilage organism and an integral part of wine/beer flavor formation. The paradox arises as the aromas generated by the yeast vary in concentration and nature

(chemistry). By altering the ratio of the aromas, we can change the perception of attributes in a wine. For example, by changing the ratios of *Brettanomyces*-generated aromas in a wine, we can observe attributes from horse sweat to Band-Aid. In addition to the ratio of the aroma compounds, the overall concentration of the compounds has an effect on perception. A low concentration of various *Brettanomyces* aromas can add complexity to a wine. As the concentration of *Brettanomyces* aromas increase, the perception of fruit decreases. At very high concentrations, only *Brettanomyces* aromas can be observed: medicinal, wet cardboard, horse sweat. The wine matrix will also impact the concentration of *Brettanomyces* aromas that will result in complexity versus off-note formation. Fruity, low tannin wines will not tolerate a very high concentration of *Brettanomyces* aromas before they are perceived as not fruity, bland wines. It is important for winemakers to become familiar with the various *Brettanomyces* aromas and their interaction in their wines. Familiarity can help to prevent winemakers from adapting to aromas that are observed in the winery on an ongoing basis. Furthermore, it is important to assess when the concentration of *Brettanomyces* aromas alters consumer acceptance of the wine. We refer to this concentration as the "consumer rejection threshold." Our goal is to identify the concentration and composition of *Brettanomyces*-generated aromas that result in a decreased liking of the wine.

Disease Control and Vineyard Practices

Production and Delivery of a Biocontrol Agent for *Eutypa* Prevention

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Research conducted on natural colonizers of grapevine pruning wounds identified the fungus *Fusarium lateritium* as a potential biological control agent for preventing *Eutypa lata*. However, little research has been done on producing and formulating grapevine colonizers, let alone *F. lateritium*, for *E. lata* control. An ideal product would maintain efficacy during storage, permit ease of application at pruning, prevent drying upon application thus promoting germination of *F. lateritium* propagules, and be physically stable. The objectives of this research were to investigate production of *F. lateritium* chlamydospores and their formulation in an invert (water-in-oil) emulsion and formulation delivery simultaneously with pruning. In an invert emulsion formulation, the emulsion traps water around the organism and slows evaporation of water once applied. These are particularly beneficial characteristics for organisms that are sensitive to desiccation and/or have a lengthy dew period. A set of formulation parameters was identified that allows simultaneous application of *F. lateritium* with pruning. The physical stability and efficacy of formulated *F. lateritium* chlamydospores improved as the percentage of water in the emulsion increased. However, too much water in the emulsion resulted in physical characteristics that would make the formulation difficult to apply to a pruning wound. Humectant addition also improved physical stability; however, the results showed that too much humectant had detrimental effects on the biological stability and efficacy of *F. lateritium.*

Quantitative Detection and Distribution of *Eutypa lata* in Grapevine

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Detection of *Eutypa* in grapevine is complicated by slow growth of the pathogen and delayed manifestations of the symptoms. Recognition of the disease is difficult until extensive invasion has occurred, by which time it is usually too late for effective remedial surgery. Our objectives were to develop a quantitative nucleic acid-based procedure to detect *E. lata* in grapevine tissue and to use the procedure to document the distribution in infected wood. The procedure involves DNA extraction directly from wood tissue using a commercially available DNA extraction kit and PCR amplification of *E. lata* DNA using *E. lata*-specific PCR primers in a real-time quantitative sequence detection system. The procedure is performed relatively rapidly in a single day, in comparison to the current method of surface disinfecting wood chips, plating wood chips on media, isolating a possible *Eutypa* colony, and then either waiting for the cultures to conidiate or performing PCR to identify *E. lata*. Our method allows detection in a variety of samples, including the surface of pruning wounds, which are grossly contaminated and consequently poorly suited for plating, and grapevine cankers, which actually contain multiple organisms that might outcompete *Eutypa* during plating. Using the quantitative nucleic acid-based procedure, we measured the concentration of *E. lata* within cankers and beyond the leading edge of canker margins in asymptomatic wood in several different varieties of grapevines. Although *E. lata* was detected in asymptomatic wood, it was only detected 4 cm from the canker margin.

Existence of Intact Xylem in Postveraison Grape Berry

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Previous studies on several grape (*Vitis vinifera* L.) varieties (Muscat Gordo Blanco, Riesling, Pinot noir, Shiraz, and Cabernet Sauvignon) with anatomical and dye-infusion techniques have been interpreted to indicate that the xylem conduits within the berry lose integrity and become dysfunctional at or upon the onset of ripening (veraison). We reevaluated xylem functionality in these berries by repeating the dye-infusion experiments and by employing a wick or a pressure membrane apparatus (PMA) to introduce the apoplastic tracer dye, basic fuchsin (0.1%), into the berries through the excised pedicel. Feeding the dye by dipping the pedicels in dye resulted in dye infusion into the peripheral and axial xylem vasculature of preveraison berries, but there was no such dye movement in the postveraison berries. However, upon introducing the dye by the wick method or PMA, the red color appeared throughout the

axial and peripheral xylem strands in postveraison berries of all five varieties. The results demonstrated that the xylem conduits remain intact and functional in the postveraison berries. Thus, some other factor decreases the driving force or increases the resistance to sap flow in postveraison xylem.

Correcting a Breeding Mistake and Discovering Novel Sources of Resistance to *Xiphinema Index* and Pierce's Disease

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For 15 years, the Walker lab has been evaluating *Vitis rupestris* x *Muscadinia rotundifolia* (rupxrot) selections for resistance to *Xiphinema index* (dagger nematode) and more recently Pierce's disease (PD). Very strong resistance to this pest and disease exists in these selections. In addition to breeding efforts, populations from these crosses have been used in genetic maps, most notably 9621, a cross of two rupxrot selections, D8909-15 x F8909-17. Genetic mapping efforts recently began incorporating SSR DNA markers to improve upon our AFLP marker-based map. SSR markers clearly define parentage, and, when applied to the 9621 population, they indicated the parents were not rupxrot selections. This discovery led to testing of all possible male parents surrounding the *V. rupestris* parents of all the "rupxrot" progeny and the determination of the true source of *X. index* and PD resistance. These crosses were inadvertently outcrossed with wind-blown pollen from nine *Vitis* species collected in Mexico by H.P. Olmo, in addition to the applied pollen from *M. rotundifolia* cultivars. As a result, novel sources of very strong resistance to *X. index* and PD in *V. arizonica* and to PD in *V. arizonica/V. candicans* hybrids have been identified.

Botrytis Bunch Rot: Effects of Infection Timing, Cluster Architecture, and Berry N Content

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Clusters of Pinot noir clones 29 (compressed architecture), Mariafeld (loose architecture), and 29 hand-thinned to approximate the Mariafeld architecture were inoculated with *Botrytis cinerea* spores at bloom, pea-sized berries, bunch closure, and veraison. The percentage of berries with latent infections, typically 70 to 90%, was the same for all three clonal treatments at any inoculation date. Most latent infections remained inactive, and there was no relationship between their frequency and the frequency of rotten berries at or after harvest. Inoculations at veraison produced much more disease than did inoculations at any other time. When 29 clusters were thinned to resemble those of Mariafeld, both developed the same levels of *Botrytis*, which were substantially lower than for unthinned 29 clusters. In unthinned 29 clusters, inoculating a single berry at veraison resulted in disease spread to 50 additional, contiguous berries after harvest, whereas only three to seven additional berries became diseased in thinned clusters. Similar results were obtained with cvs. Chardonnay and Riesling, where preharvest urea sprays also increased disease spread from such point sources. These results suggest that severe *Botrytis* losses result from berry-to-berry spread after fruit become highly susceptible postveraison and that spread is exacerbated by berry contact and high N levels. Thus, the importance of latent floral infections may be establishing the pathogen in the clusters rather than causing significant direct loss. Additional results suggest that the activation of latent infections (berries become rotten) also may be promoted by both high N content and high atmospheric humidity.

Effect of Irrigation on Pinot noir Performance in the Willamette Valley

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In the Willamette Valley there is abundant soil moisture during the period of active growth and vines tend to develop a large canopy requiring a considerable amount of water. Soil water content reaches critical values during the ripening period. Three irrigation strategies were compared in a commercial Pinot noir vineyard in the Willamette Valley. Nonirrigated controls (NI) were compared to vines irrigated to replace 50% ETc (RDI, regulated deficit irrigation) on both sides of the root system and to vines irrigated to replace 25% of ETc (crop evapo-transpiration) on one side of the root system switched every two weeks (PRD, partial root-zone drying). Each treatment was replicated five times in groups of 12 vines in a complete randomized experimental design. Irrigation treatments were implemented when vines reached a midday leaf water potential of –1MPa (first week of August), and continued until midripening. Leaf chlorophyll content, stomata conductance, and stem water potential were lower for nonirrigated vines. There were no significant differences between PRD and RDI vines, even though PRD vines received only half the water of RDI vines. Photosynthesis during midripening was highest for PRD vines, followed by RDI and NI. Later in the season, both RDI and PRD vines had similar rates of photosynthesis, which were higher than NI vines. Leaf senescence, as assessed by chlorophyll content, was hastened by drought stress. There were no significant differences in yield and yield components and fruit composition. Results on wood carbohydrate content and wine composition will be presented.

Spatial Analysis of Mineral Nutrient Retention and Microbial Processes in the Drip Zone

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Despite widespread use of drip irrigation in the arid west of North America, little is understood about how this practice influences local soil properties or the distribution of mineral nutrients applied as fertilizers. Application of water to this zone occurs when precipitation events are rare and soil temperatures are high. It represents a restricted soil volume so nutrient concentrations would be relatively high even when overall rate of

fertilizer application is small. To improve our understanding of how such localized microirrigation affects soils, we undertook 3-dimensional mapping of drip irrigation zones in a Merlot vineyard. Nutrient content and microbial processes were characterized following an application of 25 kg ha^{-1} of N as potassium nitrate. Our sampling scheme consisted of 24 individual compartments at three depths. Each individual compartment was analyzed for extractable nitrate, ammonium, and potassium, pH, total organic C and N, net N mineralization potential, and other microbial processes such as net nitrification and denitrification potential. Organic C and N were diminished in the drip zone, even with respect to row centers that were clean-cultivated using herbicides. Nitrate concentration was only temporarily elevated. Nonetheless, we found substantial potential for microbial nitrogen trace gas production long after nitrate had been leached out. Thus, it appears that one important mechanism for loss of N from this soil zone is loss of nitrogen in gaseous form.

Student Viticulture Posters

Changes in Phenolics and Antioxidant Activity of Muscadine Grape Genotypes during Berry Development and Ripening

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Phenolic compounds present in all plants are of great importance for plant-derived food and drink products since these compounds are responsible for their organoleptic properties. Grape phenolics contribute substantially to the quality of wine, as they affect color, flavor, stability, and aging behavior. They also possess antioxidant activity because of their free radical trapping behavior. The antioxidant properties of these compounds have been associated with the reduction of age-related diseases in humans such as heart disease and cancer. A number of phenols are regarded as preinfection inhibitors, providing a plant with a certain degree of basic resistance against pathogenic microorganisms. These phenols provide a wide range of economic properties; therefore, knowledge of phenolic composition in grape berries will be useful to winemaking in particular and the grape-related industry in general. This has motivated and increased study of polyphenols in an attempt to establish the phenolic fingerprinting of cultivars and general vineyard management. The aim of this study was to determine total phenolic compounds and antioxidant activity of 15 Muscadine grape genotypes and to determine their genetic variation in total phenolic content and antioxidant activity during berry development. Berries were harvested from the Florida A&M University viticulture center and cut open into skin, pulp, and seed from which phenolic compounds were extracted. Statistical analysis showed a significant variation in total phenolic content between the genotypes and accumulation level in the order of seed>skin>pulp. More information will be provided once all collected data are computed and analyzed.

Segregation of Resistance to Root-Knot Nematodes in a *Vitis mustangensis* Hybrid Population

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Development of rootstocks resistant to root-knot nematodes (*Meloidogyne* spp.) is a priority in grape breeding. The *N* allele, present in the rootstocks Dog Ridge, Ramsey, 1613C, Harmony, and Freedom, confers resistance to *N*-avirulent strains of *Meloidogyne incognita*. Extensive planting of rootstocks containing the *N* allele has led to the development of nematode strains virulent on *N*, prompting a search for new resistance alleles effective against these *N*-virulent nematodes. The nematode resistance of a seedling population derived from a *Vitis mustangensis* hybrid was evaluated to investigate the genetic control of resistance in this species. The female parent of the population was the phylloxera-resistant rootstock 161-49C, a *V. riparia* x *V. berlandieri* hybrid. 161-49C does not contribute resistance to root-knot nematodes to its progeny. The male parent was a selection of *V. mustangensis* x *V. rupestris* from the USDA grape breeding program in Parlier, CA. This selection demonstrates resistance to *N*-virulent nematodes, but the genetic nature of its resistance was not known. The source of resistance in the *V. mustangensis* x *V. rupestris* selection is its *V. mustangensis* parent. *V. mustangensis* is native to eastern Texas and parts of Oklahoma, Louisiana, and Arkansas and is very difficult to root from cuttings, essentially precluding its direct use as a rootstock. Resistance in the 161-49C x (*V. mustangensis* x *V. rupestris*) cross was assessed in greenhouse pot culture. Seedling roots were stained in an eosin-Y solution six weeks after inoculation with approximately 1500 second-stage *N*-virulent *Meloidogyne arenaria* juveniles. The resistance classes of 92 seedlings were determined by assessing the degree of galling and number of egg masses per root system. Segregation in the seedling population was consistent with a 1:1 ratio of resistance to susceptibility, indicating that the *V. mustangensis* x *V. rupestris* selection is heterozygous for a dominant allele conferring resistance to *N*-virulent root-knot nematodes. The genetic relationship between this allele and the *N* allele has yet to be determined.

Proteomic Analysis of Grapevine Tissues from Siblings Segregating for Resistance to Pierce's Disease

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By studying protein expression profiles (proteome) of Pierce's disease resistant vines, grape proteins involved in resistance may be identified. The 9621 population derived from a cross of D8909-15 x F8909-17 and its male (F8909-17) appears to have a single locus for resistance to *Xyllela fastidiosa*, the bacterium that causes Pierce's disease (PD). One hundred eighty-one individuals were screened for PD resistance from this population. As a result of this testing, the four most susceptible and the four

most resistant plants were selected to have their proteomes analyzed and compared. Comparisons of proteins from susceptible and resistant plants can determine differences associated with resistance. The four resistant and four susceptible progeny were bulked into two samples. Stem tissue of approximately 4-cm in length was ground and prepared for first dimension isoelectric focusing gel. After the strips were actively rehydrated and the sample incorporated onto the gel, isoelectric focusing commenced, and the proteins separated by their isoelectric point. The gel strips were then placed on SDS polyacrylamide gels and separated by molecular weight. The resistant and susceptible plant gels were analyzed by image analysis software, and the proteins not shared by the resistant and susceptible bulks identified by mass spectrometry. By identifying the noncommunal proteins, it is possible to determine which proteins may be involved in resistance to *X. fastidiosa* and which are artifacts of sibling distinction. Preliminary analysis of this data found unique proteins were expressed and further characterization is underway.

Phenotypic Segregation of Grape Phylloxera Resistance in Grapevines

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Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), is an important insect pest of grape and continues to impact the world's vineyards. It is native to North America but has spread to every region where grapes are grown, causing billions of dollars in damage. Rootstocks, bred from resistant North American grape species, have been used to control phylloxera for over 100 years. However, some, such as AXR#1, have failed, with catastrophic costs to the wine industry. To prevent another widespread failure, genetic mechanisms underlying grapevine response to phylloxera must be understood. This is the first study to address genetic control of galling, which indicates susceptibility. Eighty-two F_2 progeny from a remake of AXR were screened with grape phylloxera originally collected on AXR#1 roots from Napa, Sonoma, and Mendocino counties. Total numbers of both tuberosities and nodosities, which are positively correlated with susceptibility, were recorded. Chi-square analyses showed that at least two genes are involved in nodosity segregation, but one or more genes are responsible for tuberosity segregation. DNA marker analysis on this hybrid grape population is in progress to correlate AFLP and SSR markers with resistance and susceptibility and to create a linkage map. These markers will be useful in resistance breeding and serve as the basis for more detailed studies of resistance mechanisms in *Vitis*.

Evidence for More Aggressive Phylloxera Strains

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We hypothesize that the rootstock *Vitis berlandieri* x *V. riparia* Teleki 5C is not highly resistant to grape phylloxera. In order to test this hypothesis, we obtained phylloxera isolates from 10 Hungarian viticultural districts and tested their ability to develop on Teleki 5C in laboratory root-bioassays. The strains were evaluated in terms of survivorship, development, and reproduction over a 4 to 6 week period on root bioassays. Root bioassays were conducted on *V. vinifera* Chardonnay, Teleki 5C, and the *V. berlandieri* x *V. rupestris* rootstock Georgikon 121. Strains originating from Villány and Eger had higher reproduction on Teleki 5C (average production of 201 and 119 eggs) and were more aggressive than the others (average production of 10 eggs) in 2002. In 2003, the Badacsony and Eger strains had significantly greater egg production on Teleki 5C. The Eger strain had very high levels of survival, development, and reproduction on Teleki 5C, which may indicate adaptation.

Root Efficiency of Various *Vitis* Species and Implications for Rootstock Breeding

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The formation of adventitous roots allows the propagation of grapevine by cuttings and grafted plants. Production of high-quality plants is dependent upon good root initiation and function, which influences top growth, yield, and berry quality. We compared the root development of 18 genotypes, including accessions of *Vitis* species used in commercial rootstocks and in breeding. Two-node green cuttings were mist propagated and once rooted, planted into 11 plastic pots filled with loamy soil. After 6 months, root and shoot dry weights were measured and root length was measured with a COMAIR root scanner. Shoot:root ratio was also measured. Large differences were observed in root weights, shoot weight, and root length. A more effective root system was associated with small root weights and large shoot weights, while larger root systems with limited shoot weights were judged less efficient. The most efficient root systems were those found on *Vitis rufotomentosa*, *V. Shuttleworthii*, and *V. simpsonii*. The least efficient were those found on *V. labruscana*, *V. Monticola*, and *V. vulpina.*

Efficacy of Vesta as an Organic Soil Inoculant for Control of Armillaria Root Disease of Grapevines

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The objective of our research was to determine efficacy of Vesta, an organic soil inoculant, for control of Armillaria root disease of infected grapevines. In 2003, we initiated a field experiment in two *Armillaria*-infested California north coast vineyards. Experimental treatments were arranged in a split-plot design with Vesta treatment as the mainplot and vine status as the subplot. Treatment was applied to entire vineyard rows via the drip-irrigation system. Nontreated rows received water. Healthy and symptomatic vines were randomly selected within treated and nontreated rows for data collection. Treatment efficacy was assessed in terms of improvements in yield and growth parameters of vines that received Vesta. As we previously found that symptomatic vines with Armillaria root disease have significantly fewer clusters, lower yields, lower cluster weights, and lower pruning weights than healthy vines, we assume that increases in these parameters among treated vines demonstrate

treatment efficacy. Treatment was applied at budbreak, anthesis, 15% veraison, and 80% veraison. Treatment increased yields ($p = 0.0757$) and cluster weights ($p = 0.0177$) of symptomatic vines in one of two vineyards examined. Treated, symptomatic vines maintained the same high cluster weights as healthy vines. Analysis of soil nutrition among data vines showed that vines treated with Vesta had significantly higher % N ($p = 0.0306$), % C ($p = 0.035$), and CEC ($p = 0.021$). Enhanced soil nutrition, increased populations of antagonistic soil microbes, or improved water relations may contribute to efficacy of Vesta.

Influence of Irrigation Management on the Depth of Mineral Nitrogen Uptake by Mature Grapevines

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Many cool-climate vineyards of southeast Australia have naturally fertile soils with high N-mineralization potential in the top 20 cm. We hypothesized that readily available mineral N contributed to excess vigor in these vineyards. An experiment was set up to determine the proportions of the seasonal vine N uptake derived from different soil depths and whether these proportions could be manipulated by controlling soil water contents through irrigation. The aim was to minimize N uptake from the high-mineralizing A horizon. Soil around irrigated and nonirrigated Sauvignon blanc vines at Whitlands, NE Victoria, was injected at 15, 45, and 75 cm depth with ^{15}N-labeled $(NH_4)_2SO_4$ solution mixed with N-Serve. The fertilizer was applied at flowering, and leaf samples taken afterward at 1, 2, 3, 4, 6, and 8 weeks for analysis of total N and ^{15}N. Harvested berries were analyzed for %N, ^{15}N, and fruit quality. Fruit quality improved in the nonirrigated vines. Leaf %N declined with time and did not show any significant effect of irrigation. The rate of ^{15}N uptake was highest from the 15-cm depth of the irrigated treatment from 4 weeks onward. Cumulative uptake of fertilizer-derived N was significantly higher for the irrigated vines, and most of this uptake occurred from 15 cm. Grapes from the irrigated vines with fertilizer placed at 15 cm had the highest fertilizer-derived N at harvest. Nonirrigated vines did not show the same effect. These results suggest that most of the vine seasonal N uptake occurred from the top 15 cm of soil and that keeping the A horizon of these fertile soils as dry as possible can reduce N uptake and hence control excess vigor.

Open-top Chamber for Measuring Whole-Vine Photosynthesis in the Vineyard

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Photosynthesis contributes directly to yield and quality of grapevines. Environmental variables such as photosynthetically active radiation (PAR), temperature, nutrition, water status, and canopy architecture have all been shown to affect grapevine photosynthesis. Therefore, measurements of whole-vine photosynthesis can reflect the response of grapevine to effects of management practices imposed on the vineyard. An open-top chamber (10 m^3) was constructed from Mylar for the measurement of gas exchange (CO_2) from a whole grapevine. The chamber was designed to accommodate two different trellis systems while minimizing any artifacts caused by the chamber itself during measurements. Ambient air was forced into the open-top chamber using a 0.1865 kw cage fan through a 120-cm long conduit. The open-top chamber was fastened onto a 60-cm wide base accommodating the vine trunk and the conduit. Laminar flow inside the open-top chamber was achieved by placing a deflector on the conduit; ambient air was forced around the canopy via three strategically placed cage fans with 12-cm diameter fan blades. Vine canopy (three locations) and ambient air temperature (one location, 180 cm above vineyard floor) were monitored using copper-constantan thermocouples attached to a data logger. A ceptometer was used to measure the PAR above the canopy, inside and outside the open-top chamber. Four chambers have been used to measure net whole-vine photosynthesis in three different vineyards at four instances during the growing season in southern Illinois. The feasibility of using an open-top chamber for understanding the response of grapevines during the growing season to various cultural practices will be addressed.

CPPU Increases Berry Size and Delays Maturation and Color Development of Table Grapes

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CPPU, forchlorfenuron or N-(2-chloro-4-pyridyl)-N'-phenylurea, is a synthetic cytokinin that has significant physiological activity on many fruits, including grapes. When applied shortly after fruit set, CPPU stimulates cell division and cell elongation, resulting in significant increases in berry size. CPPU applied during this period also delays fruit maturation, slowing the accumulation of both sugar and color. Experiments were conducted in the Central San Joaquin Valley of California in 2002 to 2003 to examine the effects of CPPU on the berry growth and maturation of three commercially important table grape cultivars: Redglobe, Crimson Seedless, and Autumn Royal. CPPU was applied to mature grapevines of each cultivar alone (0, 4, 8, or 12 mg/L) or in combination with gibberellic acid (GA; 0 to 40 mg/L, depending upon the cultivar) at fruit set or fruit set + two weeks. CPPU applied near fruit set increased the berry weight of Redglobe approximately 10% compared with untreated control; vines receiving CPPU + GA produced berries that were 15% greater than the control. The berry weight of Crimson Seedless treated with 8 or 12 g/ac CPPU at fruit set was approximately 15% greater compared to the control, and significant GA X CPPU berry sizing interactions were also observed. CPPU applications on Crimson Seedless resulted in a more spherical berry shape compared to GA applied alone or the control. In contrast, CPPU and GA had no significant effects on the berry size of Autumn Royal. CPPU delayed fruit maturation and color development three to four weeks in all cultivars, but had no significant effect on vine yield components.

Rootstocks for Pinot Noir

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Rootstocks are the only practical way to overcome problems such as phylloxera, nematodes, or site difficulties. They can also control vigor, yield, and fruit composition, playing a fundamental role in overall success of vineyard operations. This trial was planted at the Oregon State University Woodhall III research vineyard in 1997. Pinot Noir was grafted to 19 different rootstocks and compared to ungrafted vines in a completely randomized block design. Physiological measurements included gas-exchange, chlorophyll content, vegetative growth, and water relations. Fruit yield and composition was also evaluated. Changes in photosynthesis, transpiration, and midday stem water potential indicated a dramatic effect of the different rootstocks. Pinot noir vines grafted to 101-14 Mgt, Börner, and Riparia Gloire suffered the highest water stress, and consequently presented lower photosynthetic rates. Across all the rootstocks a 6-fold difference in yield was observed without any significant difference in berry weight. Results on the impact of rootstocks on fruit nitrogenous compounds and phenolic profiles will be addressed. The information presented will provide growers and vintners a better understanding of the importance of rootstocks on vineyard and winery ventures.

Canopy Light Interception of Trellised Versus Matted Rootstock Mother Vines

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Trellised rootstock mother vines are assumed to have a superior canopy light environment to mat-trained vines, as a vertical trellis may provide more access to sunlight for all leaves, thus a potentially greater photosynthetic capacity. However, we found that mother vines trained to a single diagonal cord-style trellis may not have enough leaf area to use sunlight efficiently, meaning light simply passes through the canopy. This would indicate a less than ideal allocation of growing space. Canopy light transmittance values were tested for three rootstock cultivars: 110R, 420A, and 101-14 Mgt. Transmittance was found by dividing below-canopy radiation by above-canopy radiation. Vines were trained to two different trellis systems, a matted system or a single-training cord running from the trunk to a wire 7 feet high, at approximately a 45° angle. Vines were treated with nitrogen fertilizer or left unfertilized. A 3-dimensional model of light transmittance throughout the grapevine canopy was created for one typical vine of each training type and cultivar. A Decagon LP-80 PAR/LAI Ceptometer, which measures photosynthetic active radiation with an 83-cm light meter, was used for all measurements. Transmittance values were found to be significantly lower for matted vines than for trellised, indicating a greater interception of light by the matted vines. Light interception varied between cultivars, with matted 101-14 vines intercepting less light than the others. The response to nitrogen fertilization varied among cultivars, where 420A grew a smaller canopy when fertilized than when not, although the inverse was seen with 101-14 and 110R.

Student Enology Posters

Interactive Effects of Nitrogen and Biotin on Yeast Growth, Fermentation Rate, and Volatile Production

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Biotin is an essential cofactor for key enzymes involved in amino acid and lipid syntheses. While biotin and yeast assimilable nitrogen compound (YANC) metabolism are interrelated, interactions between these important nutrients that affect fermentation have not been studied. Alcoholic fermentations were induced in synthetic grape juice media. A 3 x 2 factorial design was used with nitrogen (60 or 250 mg YANC/L) and biotin (0, 1, or 10 µg/L) as variables. Fermentations were conducted at 22°C in 5-L fermentors using biotin-depleted *Saccharomyces* strains UCD 522 or EC1118. Cell growth, soluble solids, and H_2S evolution were monitored throughout the fermentation. Once fermentative activity had ceased, the finished synthetic wines were analyzed for esters, higher alcohols, and medium-chain fatty acids. Overall, the concentration of nitrogen greatly impacted fermentation rates for both yeasts, more so than biotin. However, the two strains exhibited weak growth without biotin: EC1118 continued to ferment slowly up to day 45, while fermentations conducted by UCD 522 stuck at approximately 20% residual sugars. Nitrogen x biotin interactions affected hydrogen sulfide production by UCD 522, while only nitrogen was significant for EC1118. Synthesis of esters, higher alcohols, and medium-chain fatty acids by yeasts demonstrated a complex dependency on nitrogen, biotin, and nitrogen x biotin interactions. While nitrogen content of a must is of primary importance for a successful fermentation, biotin content and yeast strain also impact the final product quality.

Winemaking Considerations for Long Hang Times

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As a result of long hang times, winemakers must contend with managing fermentations of high sugar musts, either by humidifying the musts prior to fermentation or by fermenting at high sugar levels. Humidification can possibly affect flavor development, while high-density fermentations often run the risk of becoming stuck or sluggish and must be carefully managed. Our research examined these different approaches. High-density (~30 Brix) Syrah and Chardonnay musts were fermented under different temperature, nutritional, and humidification conditions, resulting in both completed and stuck fermentations. Flow cytometry and FTIR spectroscopy were used to analyze microbiological and chemical parameters during fermentation, respectively. Subsequent sensory analysis yielded information on the flavor impact of the different approaches, providing insight on how to contend with extremely sweet fruit.

Detection of *Oenococcus oeni* in Chardonnay and Cabernet Sauvignon by Flow Cytometry

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Malolactic fermentation conducted by two strains of *Oenococcus oeni* in Cabernet Sauvignon and Chardonnay wines was monitored by flow cytometry. Polyclonal antibodies specific for *O. oeni* were developed in rabbits and were used in the detection assay. A fluorescent secondary antibody was used to obtain fluorescence gating of *O. oeni* cells in addition to gating cells by light scattering. Liquid counting beads were added to processed samples to obtain cell number. The time from the collection of the tank sample to running the sample on the flow cytometer is approximately 45 min.

Assessment of the Importance of Aquaporins in Wine Yeast Fermentation

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Aquaporins are water-selective channels that facilitate rapid water flow across cellular membranes and are important membrane components in a wide range of organisms. Two aquaporins (*AQY1* and *AQY2*) have been identified in the yeast genome, and, surprisingly, different laboratory and wild-type strains of *Saccharomyces cerevisiae* were found to have specific sequence and functional differences in these genes. Most laboratory strains, including the *Saccharomyces* genome database strain (S288C), have been shown to contain nonfunctional alleles of both aquaporin genes, while some wine strains (such as UCD932) have been shown to contain at least one likely functional allele of *AQY1*. This divergence between strains has not been sufficiently explained. The physiological role of aquaporins is not well understood even in laboratory strains, and *AQY1* function during alcoholic fermentation has not been studied in wine strains. This research aims to characterize the role these membrane proteins play in wine yeast during fermentation. *AQY1* was deleted from UCD932 by the one-step gene-replacement method using the geneticin resistance cassette *KanMX*. This *aqy1* mutant was then tested against the wild-type strain in competition assays under varying fermentation conditions. No major difference in enological aptitude was found at three different nitrogen levels, at 35°C, or at 26 Brix. The mutant also did not show a significant decrease in cell viability when shocked with 16% ethanol compared to wild type. Further studies will include Northern blot analysis to study *AQY1* expression and altering the timing of *AQY1* expression during fermentation.

Tracking Glucose Adaptation in *Saccharomyces cerevisiae*: The Hexokinase PII Story

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In its native environment of grape juice, *Saccharomyces cerevisiae* is able to utilize hexoses efficiently over a 1,000-fold concentration range. In some cases, the yeast fail to utilize all available sugar resulting in an unfinished fermentation. Adaptation to the use of low substrate concentrations involves regulatory proteins such as the enzyme hexokinase PII, encoded by the *HXK2* gene. Yeast lacking hexokinase PII did not show a significant growth defect when grown in both high (2% w/v) and low (0.05% w/v) glucose under laboratory conditions. However, upon shift to low glucose, a null mutant of *HXK2* showed a significantly faster rate of glucose utilization than the wild type, suggesting that the regulatory role of *HXK2* must be relieved in order for efficient use of glucose at low substrate concentrations to occur. This observation has impact under winemaking conditions as completion of fermentation is thought to require a shift from *HXK2* to *HXK1*expression. DNA array analysis was used to evaluate the regulatory role of *HXK2* during shift of cells to low substrate. Known glucose repressible genes generally fell into one of three main classes: fully derepressed in the *hxk2* null in high glucose; partially derepressed in the *hxk2* null at high glucose with expression increasing upon shift to low substrate; and repressed and derepressed independently of the *hxk2* allele. Genes requiring Hxk2p for maximal levels of expression were also identified. These genes all contained putative Med8p-binding sites in their regulatory regions, confirming the potential importance of these sites in Hxk2p-mediated glucose control.

Analysis of Acetic Acid in Stuck and Sluggish Fermentations

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Acetic acid at concentrations from 0 to 4 g/L were added to a synthetic grape must media at 22 and 28 Brix, followed by inoculation with two different strains of *Saccharomyces* yeast. Acetic acid was shown to cause extended fermentation lag times, decreased rates of fermentation, decreased and delayed development of biomass, and inhibition toward the uptake of amino acids during nitrogen metabolism. The decreases in fermentation rate were the result of decreased biomass rather than decreases in fermentation activity per cell. The critical level for a difference between control and test samples was determined to occur between 1.5 and 2.0 g/L of acetic acid added at the start of fermentation. Concentrations of acetic acid around 3.0 g/L consistently produced a stuck or sluggish condition in the experiments performed. High Brix samples were noted to compound the difficulties witnessed at low Brix levels, and in particular resulted in more instances of stuck or sluggish conditions at lower levels of acetic acid. The increase in stuck and sluggish fermentations at high sugar was attributed to higher levels of alcohol that had a co-inhibitory effect along with the acetic acid. A loss of viability by the yeast was not witnessed, even at 4.0 g/L acetic acid. The impact of acetic acid was not a key factor in the incidence of stuck or sluggish fermentations in wines, as levels required to cause consistent problems in fermentation were well above levels normally occurring in wine.

Detection and Enumeration of Wine-Related Bacteria Using Real-Time PCR

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A number of molecular biology techniques have been developed to identify the indigenous bacteria in commercial wine fermentations. The majority of these methods identify microorganisms after isolation on plates. Unfortunately, traditional plating and enrichment procedures are time consuming and often miss populations for which enrichment is problematic. More importantly, delays in identification of spoilage bacteria, such as *Acetobacter* sp., can postpone corrective action by winemakers and risk product integrity. In order to quickly and definitively enumerate different bacterial populations present in wine, we developed two real-time PCR methods; one assay that is specific for lactic acid bacteria and another assay that encompasses both lactic acid bacteria and acetic acid bacteria. Both PCR assays do not amplify yeast or other eukaryotic DNA found in the wine environment. Real-time PCR assays were designed using the SYBR green real-time PCR chemistry and primers specific to bacterial 16S rDNA sequences. Each population was enumerated by comparison to standards, and the assays were not impacted by inhibitors present in grape juice or wine. Moreover, each assay was designed so that the target population could be enumerated in the presence of a 10,000-fold greater concentration of *Saccharomyces cerevisiae*, the dominant yeast in winemaking. The creation of real-time PCR techniques will enable a better understanding of the ecological changes that occur during wine fermentations and will provide winemakers with more rapid and reliable diagnostic tools to prevent spoilage.

ELISA Screening Assay for Identifying Mold Contamination in Cork Closures and Correlation with TCA Level

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Several species of *Aspergillus* and *Penicillium* are believed responsible for the methylation of 2,4,6-trichlorophenol, forming the corresponding chloroanisole. Detecting the presence of these molds may be a quick, reliable method of determining if cork closures are tainted with 2,4,6-trichloroanisole (TCA). Uncoated cork closures were soaked for 24 hr in Chardonnay wine and analyzed for the presence of TCA via gas chromatography-mass spectrometry (GCMS) using a solid-phase microextraction (SPME) fiber and then tested for the presence of *Aspergillus* spp. and *Penicillium* spp. via enzyme-linked immunosorbent assay (ELISA). Strong positive correlation was found between the presence of TCA and the molds. The ELISA assay may provide a rapid screening tool for identifying corks that may be potentially tainted with TCA.

Influence of Ripening and Harvest Timing Decisions on Factors Affecting Stuck and Sluggish Fermentations

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Wine fermentations that are slow to complete or fail to complete the conversion of sugars to alcohol are known as sluggish and stuck fermentations and are a chronic problem in the commercial wine industry. Harvesting at higher sugar levels leads to higher ethanol concentrations and exacerbates the problem of slow or incomplete fermentations. Previous research in our laboratory has identified several juice components that play a critical role in determining fermentation kinetics. The objective of this research is to track these critical components in immature grape berries throughout the ripening process from veraison to harvest. Berry samples were taken from four commercial Chardonnay vineyards in northern California and the University of California, Davis vineyard in Davis. Sampling began at veraison and was continued until harvest 8 to 15 weeks later. Various organic acids, amino acids, sugars, vitamins, and metals were measured in each sample. Patterns and general trends will be presented for each of the key juice components, as well as the implications of these results in harvest timing decisions and fermentation management.

Development of Flavor/Flavor Precursor in Pinot noir Grapes

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Although Pinot noir is increasingly popular in the United States, it is still one of most difficult grapes to grow and to make into fine wine. The objective of this experiment was to investigate the key flavor and flavor precursors formed during berry development in Pinot noir grapes, which will help in understanding their relative importance in wine flavor and wine quality. Oregon Pinot noir grape samples were collected during the 2002 and 2003 growing seasons. Using both purge-trap and solvent extraction, crushed berries were analyzed individually. Results indicated that different aromas followed different paths during grape development, which were independent of sugar and acid development. Generally, flavor compounds have low concentrations prior to veraison. Beginning at veraison, many green odor-active flavor components, such as hexanal, *trans*-2-hexanal, hexanol, and *trans*-2-hexenol, developed rapidly and peaked. Concentrations of these components decrease during grape maturation. However, 2-methylbutanal, 3-methylbutanal, isoamyl alcohol, and isobutyl alcohol continue to increase though harvest. As characteristic floral and fruity aroma compounds, geraniol, phenol, and phenylethyl alcohol sustained low concentrations and showed little change during overall grape development. Since free forms are not the only source for those compounds in wines and many glycoside compounds were hydrolyzed during winemaking, the development of those precursors was also discussed.

Pinot noir Wine Aroma Analysis by Sequential Ion Exchange and Normal-Phase Chromatography-Gas Chromatography/Olfactometry

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Although some research has been conducted to identify odor-active compounds in Pinot noir wine, its aroma profile is complex and not completely understood. This research fractionated aroma compounds in Oregon Pinot noir wine using sequential ion exchange and normal-phase chromatography and analyzed the compounds using a gas chromatography/olfactometry mass-spectrometry technique. Aroma compounds were extracted and fractionated into acidic and nonacidic fractions on an amino ion exchange cartridge. The nonacidic portion was further fractionated on a silica gel column into four fractions: pentane, pentane-ether (95:5), pentane-ether (90:10), and ether. Gas chromatography/olfactometry (OSME) was performed on all fractions. Based on the OSME value, the most important aroma compounds in the acidic fraction were hexanoic, 3-methylbutanoic, 2-methylpropanoic, and acetic, benzeneacetic, and butanoic acids, which mainly contribute to cheesy and rancid notes. The most important esters were ethyl hexanoate, isoamyl acetate, ethyl 3-methylpranoate, ethyl 3-phenylpropanoate, ethyl 3-methylbutanoate, phenylethyl acetate, and ethyl butanoate. Many alcohols were identified as contributing to Pinot noir aroma: isoamyl alcohol and isobutyl alcohols are the most important wine alcohols; benzeneethanol and benzyl alcohol give rosy, floral aromas; 1-hexanol and *cis*-3-hexenol are responsible for green, fruity notes; methionol gives a vegetable note. Other minor aroma compounds were also detected.

Phenolic Characterization of Oregon Pinot noir Wines: An Examination of Regional and Sensory Differences

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The Willamette Valley American Viticultural Area (AVA), designated in 1984, encompasses 1.3 million hectares and is the principal region for Pinot noir wine production in Oregon. In 2003, proposals for creating six new AVAs within the Willamette Valley were announced based on the idea that distinct differences exist among the wines from these subregions. We conducted a phenolic survey of 150 commercially produced vineyard-designate Pinot noirs to determine whether significant differences could be found in the phenolic composition among the proposed AVAs. The proposed McMinnville AVA had significantly higher mean total tannins than the other proposed AVAs, while the mean total tannins for the proposed Chehalem Mountain AVA was significantly lower than the other proposed AVAs. When compared to a similar survey of California Pinot noir wines, the range and mean total tannins for Oregon was indistinguishable. We further examined the impact of site within a single vineyard by measuring the phenolic composition of fruit and wines from two different blocks in the same vineyard produced under the same winemaking conditions. While no significant differences were found in the phenolic composition between blocks, in the fruit or pomace, the resulting wines differed in their total tannin content by a factor of two. Additionally, sensory evaluation of the survey wines was performed to determine the correlation between perceived astringency and total tannin content as measured with a protein precipitation assay. We found significant correlation ($p < 0.05$), indicating that the protein precipitation assay may be a convenient way to analytically predict perceived astringency.

Effects of Closure Type on Consumer Perception of Wine Quality

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There has been much discussion and speculation regarding alternative packaging in the wine industry as it relates to the consumer. However, little research has been conducted in this area. The purpose of this sensory research was to determine consumer perception of wine quality as it related to three types of wine bottle closures: natural cork, synthetic cork, and screw-cap. Two commercial Chardonnay and Merlot wines were used for this study. Through a series of difference tests, it was determined that there was not a statistically significant difference among wines bottled in each of the three types of closures. In a second study, consumer liking and quality scores were assessed; first without any knowledge of the closure type (the first session) and then one week later with knowledge of the closure type through the use of photographs (the second session). The rating scores from the two sessions were compared to determine the effect of closure type on consumer quality perception. The difference between the first and second sessions liking scores for the three types of closures did not significantly differ (Chardonnay: $F = 1.72$, $p = 0.18$; Merlot: $F = 1.46$, $p = 0.233$). However, the difference between the first and second sessions quality scores for the three types of closures did significantly differ (Chardonnay: $F = 11.48$, $p < 0.01$; Merlot: $F = 5.06$, $p < 0.01$). There was little difference in the consumer quality scores without and with knowledge about the closure for both Merlot and Chardonnay for the natural and synthetic corks. The quality scores decreased after learning the closure was a screw-cap for both Merlot and Chardonnay.

Oxygen Induces Free Radical Formation in Red Wine

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Reactive oxygen species are known to participate in oxidation reactions in wine. When these react with catechol derivatives, semiquinone radicals and quinones are formed which then react with other polyphenols to condense and polymerize. To better understand these reactions in wine systems, and in a project studying the effects of microoxygenation on wine phenolics,

we developed a series of experiments to determine the presence, origin, and activity of free radicals in red wine using electron paramagnetic resonance spectroscopy. When concentrated wine samples were saturated with O_2, high free radical signals were obtained, but in the presence of nitrogen, none were detected. Finally, when wine phenolic extracts were studied, similar but cleaner signals were acquired. This data shows that oxygen in wine can lead to the formation of phenolic free radicals, reactive intermediates thought to lead to the formation of polymeric structures related to tannins. Future research will focus on the evaluation of the radicals' stability and their reactions with other wine substrates. These studies will help explain the chemical changes induced by low-level oxygen treatment of wine.

Development of Anthocyanins and Tannins in Pinot noir Grapes and Wine

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Phenolic compounds were monitored over three consecutive vintages to understand tannin and anthocyanin development and composition in Pinot noir grapes (Pommard clone) and to begin to understand the extent to which vineyard and winery practices might be used to influence their composition. Data was collected during the 2001 through 2003 growing seasons. Grapes were grown at the Oregon State University experimental vineyard located in Alpine, Oregon. Five cluster samples (x5 replicates) were collected for analysis each week beginning approximately four weeks prior to veraison and continued through commercial harvest. For wine production, grapes were picked at three different maturities. These three harvest dates were designed to investigate the extremes of what industry might observe. Wines were made in accordance with commercial practices and were also analyzed for phenolic composition. At harvest time, 2003 had the lowest concentration of flavan-3-ol monomers in seeds. However, the concentration of tannins was the highest in comparison with the other years. Similarly, tannins in skins had the highest concentration in the 2003 vintage. Anthocyanins did not vary considerably across vintage. Some relationships were found between concentration of some tannin components in grapes and fresh seed weight. Wine information showed differences in total tannin concentration between the three vintages; 2003 had the highest tannin concentration in wine. Monomer and anthocyanin concentration remained fairly constant across vintage. These results suggest that changes in weather conditions between vintages are associated with changes in phenolic content in grapes.

Effects of Fermentor Design and Scale on the Phenolic Extraction Profile during Vinification of Cabernet Sauvignon

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The design (size) and scale of wine fermentors is likely to have some effect on the chemical and sensory properties of wine. However, the magnitude of these potential effects has not been reported. Our goal was to systematically evaluate the magnitude of the effect of several fermentor designs and their subsequent extraction of phenolic components. The fermentor designs were categorized as differences in size (overall working volume), geometry (height to diameter ratio), temperature control device (for example, jacket, waterbath, ambient air), cap management method (punch down versus pump-over), and cap management intensity (volume pumped over at each event). During the 2003 harvest, fermentors at five scales ranging from 1 to 10,000 gallons were used to produce Cabernet Sauvignon wines from a single, commercial vineyard block. From samples taken during fermentation, the concentration of phenolic components was measured in order to create a profile of extraction. Total phenolics, tannin, and anthocyanin concentrations were measured by the Harbertson-Adam's assay; representative flavonoids and nonflavonoids by HPLC; and the components of color—anthocyanin, polymeric pigment, and copigmentation—by a modified Somer's assay. In addition, fermentation kinetics and temperature gradients within fermentors were measured. Based on the results from these studies, the most critical factors in fermentor design and scale will be presented. This type of knowledge will be critical both in understanding the effects of these parameters on wines produced in a winery and in applying the results of small-scale experiments at a commercial scale.

Viticulture Posters

Response of Autumn Royal Table Grapevines to Different Levels of Pruning

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Autumn Royal is a black seedless variety developed and released by the USDA-ARS in 1996. It produces large clusters with a naturally large berry size (8 to 9 g/berry) and matures mid-late season in the central San Joaquin Valley. Research on Autumn Royal was initiated in 1993 when it was tested as A97-68. The experimental plot was set up in a 5-acre-block established in 1999 at the California State University, Fresno vineyard. The purpose of this study was to determine the effect of pruning level (predetermined number of nodes per vine) on yield and fruit quality. The experimental design was a randomized complete block with three treatments, six replicates per treatment, and four vines per replicate. All vines were trained to a quadrilateral system with 6 to 7 spur positions retained per cordon. Treatment 1 vines were pruned to single-node spurs; treatments 2 and 3 were pruned to a 2-node and 3-node spurs, respectively. Mean number of nodes per vine was 21, 49, and 63 for treatments 1, 2, and 3, respectively. Shoot thinning was done by retaining one shoot per node, including the basal-bud position. Number of clusters per vine was 29, 29, and 17, respectively. The high cluster count on vines in treatments 1 and 2 reflects a high percent budbreak of basal buds and uniform bud fruitfulness of shoots that developed from the basal buds and buds in node positions 1 and 2. However, vines that were pruned to 3-node spurs had irregular budbreak (apical dominance) and low fruitfulness of shoots that developed from node position 3. Visual

observations indicated that basal buds appeared to be more fruitful than those in node positions 2 and 3. Net yield (pack out) was 10.4, 10.9, and 7.3 kg/vine for treatments 1, 2, and 3, respectively. Therefore, increasing the number of nodes per vine by leaving long (3-node) spurs did not necessarily lead to higher crop production on Autumn Royal vines. Fruit quality was not significantly affected by the pruning treatment, possibly because all clusters were selected to meet US #1 grade.

Influence of Rootstock on Growth and Yield Characteristics of Sauvignon blanc Grapevines in the Salinas Valley

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Nine rootstocks were evaluated for six years (1997 to 2002) in a Sauvignon blanc vineyard in the Salinas Valley. The site was previously planted to own-rooted vines that were infested with phylloxera and was not fumigated prior to replanting. The soil is a Lockwood Shaly Loam with an approximate rooting depth of 1.5 m. Vines were trained to bilateral cordons on a vertically shoot positioned trellis. Vines were pruned to a combination of spurs and canes. The number of nodes left varied with vine capacity. Vine spacing was 2.1 x 3.4 m (vine x row). The experimental design was a randomized complete block with eight replications of the nine rootstocks using five vine plots. The rootstocks evaluated were 1103P, 110R, 101-14 Mgt, 3309C, 44-53 Malègue, Teleki 5C, 420A Mgt, Riparia Gloire, and Freedom. Data collected included yield, components of yield, fruit composition, and growth components. Crop yield was significantly influenced by rootstock and the six-year averages ranged from 6.78 to 13.44 kg/vine. Yield separated into three groups with Freedom, 1103P, and 44-53 Malègue being higher; 110R, 101-14 Mgt, 3309C, and Teleki 5C being intermediate; and 420A Mgt and Riparia Gloire the lowest. Higher cluster number and weights were the factors most influencing crop yield. Pruning weights ranged from 0.36 to 1.37 kg/vine, with 1103P and Freedom having higher weights and 420A Mgt the lowest. Fruit composition was significantly affected by rootstock due to crop load and canopy differences.

Negative Correlation of Basal Node and Cluster Numbers in Grapevine Cultivars and Wild Species

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Grapevine shoots have nodes without clusters (inflorescences) basal to a zone in which leaf-opposed clusters are found at the nodes. Distal to the cluster zone leaf-opposed tendrils are borne at the nodes. The quantity of basal nodes and their possible relationship to clusters are important in grapevine breeding and improvement. Basal node number influences cluster position within the canopy. Cluster position helps determine fruit exposure to light, which in turn influences fruit maturation processes and management practices, including cluster treatments and harvest. Cluster number is a primary component of yield. Numbers of basal nodes and clusters were counted on 10 primary shoots each of 180 grapevine (*Vitis*) accessions. The accessions analyzed are cultivars and wild species held in the United States National Plant Germplasm System collection at the Plant Genetic Resources Unit, Geneva, New York. The correlation coefficient of the number of basal nodes and number of clusters was calculated using the means of the 10 observations per accession. Basal node and clusters numbers were negatively correlated; the correlation coefficient was -0.703, which is significant ($p < 0.001$). The negative correlation of basal node and cluster number has implications for grapevine improvement.

Grapevine Variety, Cane Numbers, and Dry-on-the-Vine Raisin Production on Overhead Arbor Trellis

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An experiment was conducted over three years to compare the performance of several raisin grape varieties (*Vitis vinifera* L.) on an overhead arbor trellis for production of dry-on-the-vine (DOV) raisins. The experiment was a split plot, with grapevine variety (DOVine, Fiesta, Selma Pete, or Thompson Seedless) as the main plot, and number of canes (six or eight) as the subplot. Fiesta produced the highest yields, averaging about 5 tons of raisins per acre. However, Fiesta generally had the lowest soluble solids and among the lowest raisin grades. The later ripening of Fiesta fruit delayed harvest pruning to initiate fruit drying compared to the other varieties. Therefore, its raisins did not always dry sufficiently to meet industry standards. Selma Pete, DOVine, and Thompson Seedless had similar yields, generally about 20% less than Fiesta, but Selma Pete usually produced fruit that were the largest in size, with the highest soluble solids, and that produced raisins of the highest grades, compared to the other varieties. Vines pruned to eight canes generally produced 10 to 15% higher yields without sacrificing raisin quality.

Row Orientation and Trellis Architecture Effects on Dry-on-the-Vine Raisin Yield and Quality

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Vine rows in traditional raisin vineyards are generally oriented east-west (EW) so that raisin trays may be placed on a south-facing terrace, in the row middles, to dry. A north-south (NS) row orientation might be preferable for dry-on-the-vine (DOV) raisins if NS rows intercept more light than EW rows. Thus, we began a study to compare the performance of Selma Pete grapevines (*Vitis vinifera* L.) in NS versus EW rows and trained to two different trellises, the open gable and the Australian-developed Shaw. After one season,

row orientation had no effect on fruit quality, yield, or drying of raisins. However, grapevines were more fruitful when planted in NS rows than in rows oriented EW. Grapevines in NS rows had more clusters on canes than did vines in EW rows, but vines in either row orientation produced a similar number of clusters on renewal shoots. However the clusters on vines in NS rows must have been smaller than those on vines in EW rows, because row direction had no effect on berry size, soluble solids, or raisin yield. Of the two trellises tested, the open gable trellis was clearly superior to the Shaw, because vines on the open gable had fewer clusters on renewal shoots and 20% greater yield than vines on the Shaw trellis.

Effect of Soil Type and Training-Pruning System on Petiole Mineral Contents at Bloom in Cabernet Sauvignon Grapevines

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A study was conducted in a Cabernet Sauvignon/Freedom vineyard planted in 1991 in Galt, CA to evaluate the effect of soil type and training-pruning system on petiole mineral contents during a period of four years (2000 to 2003). The experiment was designed as a split-plot factorial of two soil types and six training-pruning systems. Samples of 100 petioles were taken from the opposite side of inflorescence at full bloom and analyzed for major macro- and micronutrients. Columbia silt loam (a high-capacity soil) supported vines with higher content of N and Mn in all four years, Mg, Zn, and S in three years, B and Cu in two years, and Na in one year, while San Joaquin loam (a low-capacity soil) supported vines with higher content of P in all four years, K in two years, and Cl in one year. Vines trained to traditional systems (bilateral cordon, Sylvoz, and head) had higher Ca and B content in three years but lower Zn content in one year, compared to those trained to minimal or mechanical pruning. Petiole Mn content was higher in vines trained to minimal pruning than those trained to other systems. Petiole N, P, K, Mg, Fe, Cu, Na, Cl, and S contents were not influenced by training-pruning systems. Soil type did not interact with training-pruning system to affect petiole mineral contents except for Mn and Cl in 2002. The experiment demonstrated a greater effect of soil type on petiole mineral contents at bloom in comparison with training-pruning systems.

Mineral Nutrient Level Comparisons of Four Table Grape Cultivars on Ten Rootstocks in Northern Chile

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Rootstocks are not commonly used in Chile because phylloxera does not exist. However, resistant or tolerant rootstocks could be useful in areas of heavy nematode infestation or in light soils replanted after the removal of an old vineyard. It is hypothesized that rootstocks can improve cultivar vigor under poor soil conditions. However, they can also have an important effect on the mineral nutrient status of the scion. The study was carried out in four field trials using four cultivars in Elqui Valley (30°02'S, 70°44'W) over one year (2003 first results). The trials involved 2001 plantings of Thompson Seedless, Superior, Flame Seedless, and Red Globe omega grafted onto Salt Creek, Freedom, Harmony, 1103 Paulsen, Saint George, 1613C, 99R, 110R, 140R, SO4, as well as own roots. The trials were located in sites previously planted to vineyard. Experiment design was a randomized complete block with four replications. Opposite cluster petiole samples were taken at bloom and analyzed for total N, K, P, Ca, Mg, and Zn. Highly significant differences in nutrient levels due to rootstocks were found in all cultivars. Salt Creek showed the greatest incidence of significantly higher total N, P, and Zn. Saint George also showed a high total N level. Harmony and own roots were of high K and Mg, respectively. The Flame Seedless and Red Globe trials showed the most significant effects on nutrient levels. In Flame Seedless cultivar, N, P, and Zn were 1.6x, 2.4x, and 1.5x higher, respectively, and K was 2.4x lower in Salt Creek as compared to own roots. Salt Creek increased N, P, and Zn petiole concentration by 1.5x, 1.8x, and 1.7x, respectively, as compared to Red Globe own root. In the same cultivar, Saint George and Harmony had 1.7x and 1.6x higher N and K, respectively, than own root.

Grapevine Variety, Training System, and Dry-on-the-Vine Raisin Production on Open Gable Trellis

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An experiment was conducted over four years to evaluate the performance of several new raisin grape varieties (*Vitis vinifera* L.), on an open gable trellis, for production of dry-on-the-vine (DOV) raisins. The experiment was a split plot, with training system (head, bilateral, or quadrilateral) as the main plot, and grapevine variety (Diamond Muscat, DOVine, Fiesta, or Selma Pete) as the subplot. Variables, including fruitfulness, fruit composition, and raisin yield and quality, were measured. In general, vine training style did not affect fruit composition or raisin yield or quality. However, vines trained to bilateral or quadrilateral cordons produced more clusters on renewal shoots compared to head-trained vines. DOVine and Fiesta generally produced the highest yields, averaging about 4.8 tons of raisins per acre. However, Fiesta usually had the lowest soluble solids, the poorest raisin grades, and the highest field moisture at harvest. The later ripening of Fiesta necessitated a 5- to 10-day delay of harvest pruning to initiate fruit drying. This contributed to the slower drying of its fruit. In contrast, Selma Pete had among the earliest harvest pruning dates, highest soluble solids and raisin grades, and its raisins generally dried well. Diamond Muscat produced the lowest yields most seasons, but its raisins were of good quality.

Development of SSR Markers for Genotyping and Assessing Genetic Diversity in *Xylella fastidiosa*

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The objective of this work was to develop a DNA-based marker system for unambiguous differentiation and identification of *Xylella fastidiosa* (Xf) strains. Simple sequence repeat (SSR) markers are ideal because of their repeatability and precision. The genomic sequencing of four Xf strains (PD, CVC, ALS, and OLS) is now complete and this information allows the identification of repeated sequence loci. A genome-wide search was performed for SSR loci among the sequence databases of all four strains and 400 to 500 various repetitive types of SSR loci were identified from each strain. These loci were evenly distributed across whole genome and potentially useful for SSR marker design. Based on the nature and length of the repeats, 60 SSR loci were selected for primer design. We evaluated these SSR primers with five PD Xf collections; four from Napa vineyards and one from the Temecula (the one used for whole genome sequence). Results found that 70% of SSR primers were able to detect various degrees of polymorphisms among the five isolates. These PCR-based SSR markers provide an excellent tool for research in epidemiological analysis, genetic structure of microbial populations, and fingerprinting of isolated colonies.

Effect of Rootstock on Growth, Yield, Fruit Composition, and Vine Nutritional Status of Cabernet franc

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Grape rootstocks influence resistance to soilbourne pests and diseases, adaptation to soil problems, and a myriad of vine physiological processes and functions. Choice of rootstock for a site depends on complex interactions related to soil, pests, disease, water availability, and environmental and management factors. Use of rootstocks in eastern United States viticulture is increasing and more information on performance of rootstocks in the Ozark Mountain Region would be beneficial for growers. Objectives of this experiment were to evaluate the impact of selected rootstocks on vegetative growth, yield, fruit composition, and vine nutritional status of Cabernet franc grapevines. This experiment was conducted for three seasons (2000 to 2002) in a commercial vineyard near Altus, Arkansas. Cabernet franc vines grafted onto 3309 Couderc (control), 110 Richter, Freedom, and 44-53 Malegue rootstocks were planted in a Linkers fine sandy loam soil. Vineyard spacing was 2.1 x 3.1 m (vine x row), and row orientation was east-west. A four-arm Kniffen trellis system was used, and the vineyard block was not irrigated. Few statistically significant differences between rootstock treatments were observed for yield, fruit composition, or nutritional status. Vines grafted onto 3309 Couderc rootstock had lower yield in 2000 due to winter injury. Vegetative growth as indicated by dormant pruning weight was greatest for vines grafted onto 110R and Freedom. Fruit from vines grafted onto 110 Richter had lower pH as compared to fruit from vines grafted onto Freedom. Results suggest that vines grafted onto 110R or Freedom might benefit from conversion to a divided canopy due to increased vine size.

Effect of Three Rootstocks in the Bud Necrosis of Three Table Grape Varieties

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Low levels of fruitfulness due to bud necrosis have occurred in the northwest viticultural area of Mexico. This phenomenon could be increased in highly vigorous vineyards arising from the use of rootstocks. In recent years, new rootstocks have been introduced in this area. Our goal was to study the effect of three rootstocks—Harmony (HA), Salt Creek (SC), and Freedom (FR)—on bud necrosis of the main table grapes varieties in our area—Flame Seedless (FS), Perlette (PE), and Sugraone (SO). The results were compared with the fertility determined in the variety on its own roots (control). Ten dormant shoots containing three buds each were sampled in each variety/rootstock combination and in the controls. The buds were classified as necrosis, vegetative, and fruitful. The necrotic levels were lower in the control treatments compared with those varieties grafted on rootstocks. FS control had 26% bud necrosis; FS grafted on FR, SC, and HA had 43, 46, and 50% bud necrosis, respectively. SO control showed 28% bud necrosis, and SO/HA had 57%, SO/SC had 59%, and SO/FR had 46%. PE control had 10% bud necrosis; PE/SC had 60%; PE/HA had 40%; and PE/FR had 10%. Under similar commercial management of these varieties, an increase in bud necrosis is promoted by the rootstocks, requiring studies to decrease the vigor caused by the rootstocks.

Bud Fruitfulness in Vineyards from Sonora, México

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The number of inflorescences early in the season is determined by the number of inflorescence primordia that developed the previous year and by the capacity that they have to sprout after pruning. Bud fruitfulness refers to the percentage of dormant buds having inflorescence primordia. If bud fruitfulness is low, then more buds need to be left at pruning in order to ensure adequate production of bunches. The amount of inflorescences produced early in the season varies among vineyards, even when management is quite similar among them. Variation of bud fruitfulness may be the cause of such differences. The purpose of this work was to determine bud fruitfulness in several vineyards from La Costa de Hermosillo, Sonora. Bud fruitfulness of 64% of the vineyards with Perlette was from 60 to 80%, and only 7.1% of vineyards had a fruitfulness from 20 to 40%. In contrast, 40% of vineyards with Flame showed a fruitfulness from 60 to 80%. Bud fruitfulness of 28% of the vineyards with cv. Superior was from 60 to 80%, and in 28% of vineyards, fruitfulness was

from 20 to 40%. Bud necrosis of 6% of vineyards with cv. Flame was from 60 to 80%. The vineyards with cv. Superior showed the highest percentage of vegetative buds and the lowest bud necrosis. The percentage of vineyards that showed from 40 to 60% of vegetative buds was 28%, 3%, and 1% for cvs. Superior, Perlette, and Flame, respectively. Vineyards with cv. Perlette showed the highest bud fruitfulness in contrast to the ones planted with cv. Superior, which presented the lowest. Bud necrosis was highest in cv. Flame and lowest in cv. Superior. The percentage of vegetative buds was highest in vineyards with cv. Superior.

Bud Fruitfulness of Grapevines Under Greenhouse Conditions in Sonora, México

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One of the objectives in the viticulture of Sonora, México is to harvest in early May to obtain higher prices per box. However, frost damages, abcision of inflorescences, and other problems often limit harvest. Protection against low temperatures by using greenhouses may solve these problems. The objective of this work was to evaluate the effect of the environmental conditions inside the greenhouse on bud fruitfulness of the grapevines. The experiments were conducted in Costa de Hermosillo and Caborca, Sonora with the cvs. Perlette and Flame. In both sites the grapevines were drip irrigated. For Perlette, bud fruitfulness was double for natural conditions as compared with greenhouse conditions, under which bud necrosis was higher. In addition, the vegetative buds in the greenhouse were nine times higher than under natural conditions, indicating that the reduction of bud fruitfulness in greenhouse was mainly due to lower flower induction. Since relative humidity and temperature were within the optimum range, light intensity might explain the differences. Photon flux density on cloudy days was 700 µmol m^{-2} s^{-2} under natural conditions and 150 µmol m^{-2} s^{-2} under greenhouse conditions, while on sunny days photon flux density was double for natural conditions as compared with greenhouse conditions. Flame also showed lower bud fruitfulness in the greenhouse.

Changes in Internal Composition after Planting Hardwood Cuttings of Norton Grapevine (*Vitis aestivalis*)

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Changes in the levels of starch, phenolic compounds, and endogenous auxin (indole-3-acetic acid: IAA) in the cuttings of *Vitis aestivalis* cv. Norton and *V. labruscana* cv. Campbell Early were quantified and compared for 42 days after planting in a propagation bed filled with moist vermiculite. The bed was thermostatically maintained at 20°C by low-voltage heating wires. Rooting percentages at 35 and 42 days after planting were 0% and 42% in Norton and 60% and 92% in Campbell Early, respectively. Moreover, root length, number and weight per cutting, shoot length, and leaf number were significantly greater in Campbell Early than in Norton. The starch contents in the cutting of both cultivars decreased rapidly after planting, but the ratio was larger in Campbell Early than in Norton. Conversely, the contents of phenolic compounds in the cuttings of both cultivars increased gradually after planting, and it was somewhat lower in Norton than in Campbell Early at 42 days after planting. While bud removal completely inhibited rooting of cuttings of both cultivars, application of indole-3-butyric acid (IBA: 1% lanolin paste) to the position of the excised bud promoted rooting of cuttings without buds of both cultivars. The level of IAA in the cuttings of Campbell Early was significantly higher than that in Norton immediately before the appearance of adventitious roots, 26.1 µg and 7.8 µg/g fresh weight, respectively. Results show that IAA levels synthesized by the bud and decomposition speed of starch accumulated in the cuttings are closely related to adventitious root formation of cuttings.

Initial Analysis of Hybrid Varieties Blanc du Bois, Cynthiana, and Black Spanish within a Pierce's Disease Hot Zone

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Within the Gulf Coast region of Texas there are several vineyards that have planted hybrid varieties in order to survive the extremely high Pierce's disease (PD) pressure of the area. At Austin County Vineyards the varieties Blanc du Bois, Black Spanish, and Cynthiana have been grown successfully for 10 to 15 years with crop yields as high as 6.1, 3.8, and 1.8 tons/ac, respectively. Cynthiana had significantly lower crop yields in 2003 ($p < 0.001$) compared with the other two varieties. In addition, during the last 5 of its 15-year planting at this site, Cynthiana has yielded an average of 0.3 tons/ac, suggesting this variety may have limited tolerance to PD in an extreme disease area. ELISA levels from August petioles and leaf sinuses indicated Cynthiana had higher levels of *Xylella fastidiosa* ($p < 0.0001$) compared with the other two varieties. Individual leaf samples of Black Spanish and Blanc du Bois would occasionally test very high for *Xylella*, but the overall ELISA levels for these varieties was low, suggesting the bacterium may stay more localized within Black Spanish and Blanc du Bois after infection. Dilution plating and real-time PCR methods are currently underway to confirm the ELISA differences between these varieties.

Altered Root-Canopy Ratio and Its Effect on Premature Berry Dehydration of Own-Rooted Merlot Vines

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Premature berry dehydration (PBD) is a key concern for cv. Merlot growing on its own roots in the Chilean grape-growing regions and is characterized by berry shriveling just prior to

veraison, absence of color, and the appearance of odd flavors in grapes. This problem has been associated with fungal diseases, water deficits, and nutritional unbalances, but no proof of any of the above has been presented. After an extensive survey, it was determined that a common feature of the vineyards with PBD was an apparent imbalance between root and canopy development. This hypothesis was tested on three factorial field trials in which root-canopy ratios were altered by shoot trimming (30% and 50% of leaf area) or root pruning (18 days prior to veraison) and combined with three levels of water deficit. Altering root-canopy ratios resulted in lowered xylem potential and berry dehydration. The effect of water deficit does not explain on its own the PBD but can accelerate the problem. A ratio of 80 (cm leaf area /roots <2 mm diameter) is proposed as a threshold under which PBD can be avoided under standard irrigation conditions.

Effect of Temperature during Budbreak on Sap Mineral Composition and Cluster Development

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Budbreak promoter is applied to vines before their natural bud opening to obtain an early harvest, so initial development occurs under colder temperatures that could interfere with plant physiology. The effect of temperature during the early stages of shoot and cluster development was evaluated to determine the effect on mineral composition and cluster development on Flame Seedless table grapes. A group of three rows with five plants were confined in a small plastic greenhouse of 12 m wide x 8 m length x 2.5 m height and maintaining temperature from 11°C to 35°C. Another group of plants was grown under natural conditions, decreasing the minimum temperature up to 3°C. Using this greenhouse the first year, three dates of application of cyanamide (December 17, 24, and 31) were evaluated; in the second year, later dates were evaluated (December 22, 28, and January 3); and in the third year, application was limited to one date, on which three hydrogen cyanamide rates (5, 7, and 9%) were evaluated. Mineral sap concentration was evaluated on the early stages of development of shoots. A scale for cluster structure was set, from 1 for worst to 10 for best. The number of good clusters was higher in plants with controlled temperature (21) than in plants growing under natural conditions (16). Early pruning and application of hydrogen cyanamide reduced the number of good clusters. Plants with controlled temperatures had lower sap levels of nitrate, ammonium, zinc, and manganese than those growing under natural conditions.

Characterization of *Uncinula Necator* Isolates from Northern Chile According to Their Triadimefon Sensitivity and DNA Polymorphisms

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A low demethylation inhibitor (DMI) fungicide control was observed in several table grape vineyards located in northern Chile. It is hypothesized that *Uncinula necator* populations have lost their sensitivity to this group of fungicides. The study was carried out to determine the sensitivity of different *Uncinula necator* populations to DMI fungicides and DNA polymorphism analysis. Fungi-affected berries and leaves were collected during the 2001 to 2002 season in seven sites: Iglesia Colorada, Tierra Amarilla (III region), La Serena, Paihuano, El Palqui, Limari (IV region), and La Platina (Metropolitan region). Samples of *Uncinula necator* were inoculated on Muscat of Alexander plants and maintained in a growth chamber using a 24°C temperature and 12-hr photoperiod. To analyze their DMI fungicide sensitivity, heterogeneous *Uncinula necator c*ultures and subcultures were subjected to a bioessay that consisted of inoculation of disks of pathogen-free leaves previously immersed in five triadimefon concentrations (0, 0.5, 1.0, 3.0, and 10 mg/L). These disks of leaves were maintained in plastic boxes under fluorescent light for 10 days. The percentage of pathogen-colonized disks was used to determine the inhibition percentage and estimate the EC50 value by a regression model. The total of *Uncinula necator* isolations presented triadimefon sensitivity, with EC50 values ranging from 0.14 to 1.19 mg/L of fungicide. A group of isolations was subjected to a DNA polymorphism analysis to check the levels of genetic diversity among pathogen populations.

Influence of Berry Shrivel on Mineral Nutrition in Cabernet Sauvignon Grapevines

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A two-year study was conducted in three California north coast vineyards to determine the differences in tissue mineral levels related to berry shrivel in Cabernet Sauvignon grapevines. Berry shrivel is a disorder that becomes apparent close to harvest. Berries on affected clusters become flaccid while the rachis appears to be healthy (unlike bunch stem necrosis). Berries are not fully colored, and the juice tastes sour and may have off-flavors. The fruit is unacceptable to wineries and is often selectively removed prior to harvest. The cause of berry shrivel is unknown. Ca and Mg were higher in rachis tissue taken from affected vines in Oakville Experimental Vineyard (OEV) in both years and in Alexander Valley for one year. Rachis K was lower in one year at each of these sites, and the K/(Ca + Mg) ratio was lower at harvest in both sites. Total N was higher in rachis tissue from symptomatic vines in Rutherford in the one year that site had berry shrivel. In 2003, juice from affected clusters at OEV and Alexander Valley had significantly reduced soluble solids and pH and elevated titratable acidities and calcium at the onset of symptoms and at harvest. Malic and tartaric acids levels at harvest were elevated in affected fruit, while proline was reduced, indicating that there is an altered ripening syndrome in berry shrivel fruit. Distribution within the canopy was mapped and it suggests that the disorder is not related to exposure.

Regulated Deficit Irrigation Influence on Vegetative Growth and Fruit Quality in Cabernet Sauvignon

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Regulated deficit irrigation is a sustainable viticultural practice. This research was conducted for five years in an effort to control canopy growth and improve fruit characteristics of Cabernet Sauvignon through regulated deficit irrigation management. Vines were subjected to early season water-deficit stress to control vegetative growth. Once shoot elongation was reduced and the desired shoot length was obtained, the following irrigation treatments were imposed: weekly irrigation to replenish the amount of water used by the vine (control); weekly irrigation to create periodic water-deficit stress during berry cell division followed by weekly irrigation to replenish vine water usage (ED); and weekly irrigation to replenish vine water use until veraison followed by periodic water-deficit stress from veraison to harvest (VD). All treatments produced similar shoot lengths between 3 and 4 ft. The ED and VD treatments reduced berry weight compared to the control at harvest, resulting in lower cluster weights and yields. However, yield reductions were compensated for by retaining more buds during dormant pruning. No differences in Brix, titratable acidity, or pH were measured at harvest, and cold hardiness was similar among treatments. Regulated deficit irrigation in Cabernet Sauvignon consistently produced optimally sized canopies without detrimental affect on vine productivity. Short periods of water-deficit stress during the growing season improved wine quality while further conserving water resources.

Early Performance of Selected *Vitis vinifera* L. and Interspecific Hybrid Winegrape Cultivars in Arkansas

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Cultivar selection is a key component of successful and profitable viticulture. Growing the right cultivar is critical if growers are to achieve consistent production of high-quality fruit. The objective of this study was to evaluate the performance of newer *Vitis vinifera* L. cultivars. The *Vitis vinifera* L. cultivars included in this study were selected based on their reported potential to produce high-quality wine under warm-climate conditions. Selected interspecific hybrid cultivars were also evaluated. The study was conducted at the University of Arkansas Fruit Substation near Clarksville. Red and white cultivars were evaluated in separate experiments. Vines were grafted on 1103 Paulsen rootstock and trained to a vertical-shoot-positioned trellis system. The vineyard was irrigated using a drip system. White cultivars were Chardonnay (control), Chardonel, Symphony, Traminette, Verdelho, and Viognier. Red cultivars were Cabernet Sauvignon (control), Cabernet franc, Chambourcin, Malbec, Sangiovese, Syrah, and Tempranillo. Based on yield and fruit composition parameters, all white cultivars except Symphony performed well under the conditions of this study. Traminette and Verdelho had the highest dormant pruning weight among the white cultivars. Performance was less consistent for the red cultivars. Cabernet Sauvignon, Cabernet franc, Chambourcin, and Syrah performed best based on yield and fruit composition parameters. Dormant pruning weight was greatest for Cabernet Sauvignon and Malbec. These data are preliminary and further research is needed before final recommendations can be made.

Conversion of Mature Zinfandel in the North San Joaquin Valley to Mechanical Pruning

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A three-year replicated commercial field trial was set up in the northern San Joaquin Valley to observe the response of converting mature Zinfandel to two mechanical pruning systems. The control was traditional spur-pruned bilateral cordon. The second treatment was a mechanically hedged box approximately 30-cm wide and 51-cm high beginning at the cordon. The third treatment was a "V" shape similar to the Australian MPCT system, with the cut beginning at the cordon and extending 45° upward and outward. The first harvest after conversion there was a decrease in sugar accumulation because we did not reduce crop load as recommended; the plots were not harvested. The next two years the plots were harvested and at midspring low-hanging fruit was trimmed off as soon as it became pendulous. The second year the box treatment produced significantly more fruit/vine and had a slightly lower pH than the spur-pruned control or the V, but with no significant reduction in sugar or TA. The mechanical treatments had significantly less bunch rot. There was no significant difference in any of the yield components. The third year there was no difference in yield, Brix, TA, or pH among the treatments. The spur-pruned treatment had fewer clusters/vine than the mechanical treatments and much more rot than either mechanical treatment. The fourth year was not harvested, but once again we measured a significant rot reduction in the mechanically pruned plots.

Enology Posters

Genetic Diversity among *Brettanomyces* Isolated from Wines

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Brettanomyces bruxellensis has been related to sweaty, Band-Aid, spicy, mousy, and other potent off-odors in wines. To some consumers, the spicy, smoky aromas can be attractive in wines. The existence of "good" and "bad" strains has been hypothesized. In order to better understand the conditions under which this yeast can grow in wines and what flavors it can produce, it is necessary to define individual yeast strains genetically.

In this study we characterized 50 strains of *B. bruxellensis* comparing a section of the 26*s* rDNA and a portion of the actine gene. These genes had shown useful differences to allow excellent classification to yeast genus and species. The majority of the strains characterized by the portion of 26*s* rDNA are grouped into one of six clusters, two clusters contained only one strain. More differences were highlighted by sequencing the same strains for a portion of the ACT1 gene. This comparison showed eight clusters. The main group is the most similar to the ACT1 gene reference sequence. We did not find any geographically related group in the two phylogenetic trees. In other words, strains isolated in the same geographical area are not genetically similar. Combining the results of this genetic analysis with results of physiological studies might allow us to identify strains that have a positive flavor impact. It also allows us to identify strains with potentially strong negative flavor impact and allows the winemaker to intervene when necessary to protect the flavor character of a wine.

Functional Characterization of the *Oenococcus oeni* Primary Transcription Factor σ^A via Complementation

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In gram-positive bacteria the genes involved in cell maintenance are regulated by the primary transcription factor σ^A. This protein is a part of the RNA polymerase enzymatic complex and is responsible for the recognition of promoter sites. σ^A plays a key role in cell growth directing the transcription of the housekeeping genes. In a previous study, we described the operon containing the gene encoding σ^A (*sig*A) of the wine bacterium *Oenococcus oeni*. The analysis of the sequence showed that the *O. oeni sig*A gene is highly similar to the homologous genes of other gram-positive bacteria. Here, we describe a functional characterization of *sig*A of *O. oeni* by the complementation of a *Bacillus subtilis* σ^A mutant strain. The mutant contains a double amino acid substitution in σ^A that destabilizes the protein, and the mutant is unable to grow at 49°C or higher temperatures. We have cloned the *O. oeni sig*A gene into an integrative plasmid and inserted the gene into the chromosome of the σ^A mutant *B. subtilis* strain. The new strain harboring the *O. oeni sig*A gene is able to take over the mutated function and grow at 49°C. The positive result of the complementation study confirms the identity of *O. oeni* SigA and its function.

Comparison Results on the Use of Rack and Return (Delestage) with Wine Warming for Extraction of Phenolics from Pinot noir

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The influence of two different enological practices on the phenolic composition of the resulting commercial-scale Pinot noir wines was examined. Over the course of two vintages (2001 and 2002), use of rack and return (delestage) was compared with the use of wine warming during maceration for cellar-scale fermentation of Pinot noir. In addition, a control treatment was added for the 2002 comparison. Although the vineyard source was different for the two vintages, consistent patterns of phenolic extraction emerged by HPLC analysis. Total and monomeric anthocyanins and malvidin content were higher in the rack and return wines. In contrast, catechin, epicatechin, polymeric anthocyanins, and polymeric phenols were higher with wine warming. Both treatments increased the catechin, epicatechin, and polymeric phenols and decreased the malvidin, total anthocyanin, and monomeric anthocyanin contents compared with the control in 2002. The magnitude of change in the phenolics was much greater for wine warming compared to rack and return.

A Novel Rapid System for Evaluation of Polyphenolic Ripeness in Vineyards

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The goal of the research was to develop a rapid method to measure polyphenolic ripeness in the red grapes. A qualitative, optical method was proposed. Six light-emitting diodes (LED) were used to register the light signal from the grape mash. The LEDs work in the visible and infrared fields, with wavelengths from 525 to 880 nm. The method requires no extraction time, but does require mash time to prepare the samples. Analysis time is rapid (a few seconds), and numerous samples are performed per day. The signal from the LEDs was weighted according to statistical methods to produce a value number to label the maturity trend. This new index was correlated to the polyphenolic and anthocyanin indexes commonly used in wineries. The tested grape varieties were Merlot, Cabernet Sauvignon, Cabernet franc, Pinot noir, and the indigenous Italian cultivars Montepulciano, Sangiovese, Corvina, Corvinone, and Rondinella. About 40 vineyards were analyzed from four Italian and one French wine-growing areas. The determination coefficients (R^2) were greater than 0.7 for anthocyanins (mg/kg) and total phenols (Abs 280 nm) extracted with different solvents (pH 1, pH 3.2 and acidified MeOH). The ripeness trends are comparable from the optical system to other extraction methods to determine harvest date. This new system allows varietal and vineyard specific estimation of ripeness in order to decide the harvest time and builds a key database for vineyard management.

Sensory Descriptive Analysis of Traditional Madeira Wines and Wines with Added Sotolon

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Traditional Madeira wines are baked and aged. One of the aging volatiles is sotolon (4,5-dimethyl-3-hydroxy-2(5H)-furanone), which imparts an aroma described as caramel, burnt sugar, *madeirised*, *rancio*, nutty, and currylike, and which increases in concentration with age. The sensory effect of sotolon on the perceived age of Madeiras was evaluated by comparing unbaked wines with added sotolon to traditional wines. Six traditional Madeiras made with Bual grapes (1961, 1968, 1980, 1993, 1996, and 1999), and three young, unbaked Bual wines with 0, 10,

and 19 µg/L added sotolon, respectively, were compared by sensory descriptive analysis. Sixteen of 19 flavor attributes discriminated significantly among the wines. Principal component (PC) analysis showed that the first two PCs accounted for 69% of the variance. The baked and the unbaked wines were located on opposite sides of PC1. Unbaked wines were higher in musty, mushroom, and dried fruit flavors, while baked wines had more complexity of flavor and were higher in nutty, citrus, cherry, coffee, and brown sugar attributes. Correspondence analysis used to analyze the color data graphically showed a progression of increasing brownness, with increasing age up to about 23 years of aging. Although the two unbaked wines with added sotolon seemed to be more complex than the one without, this compound alone did not make them similar to the traditional Madeiras. Therefore, the baking process is important to the flavors of traditional Madeiras, and a future modification of this study would be to add the sotolon after baking but before aging.

Estimating the Rejection Threshold for 2,4,6-Tricholoroanisole in White Wine

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It is estimated that 5 to 10% of wines are tainted with 2,4,6-tricholoroanisole (TCA). Also known as cork taint, TCA produces odors described as musty, dank, or earthy that are unacceptable to many wine drinkers. Although previous estimates of TCA in wine have put the threshold as low as 0.5 parts per trillion (ppt), it is not clear at what levels TCA begins to render a wine unacceptable. It is therefore difficult, even if one knows the distribution of TCA levels in commercially available wines, to assess the economic impact of this wine taint. A method was developed to address this question by using a paired preference test within a typical method of limits threshold procedure. The aim was to determine the point at which wine consumers would begin to reject a wine containing TCA, which we termed the consumer rejection threshold (CRT). Fifty-eight regular white wine consumers (Ss) received pairs of samples of white wine and were asked to taste each and indicate which sample was preferred. Ss received replicate series of eight pairs, in which one wine sample was "spiked" with TCA at the following concentrations: 0, 0.5, 1, 2, 4, 8, 16, and 32 ppt. To determine if the CRT was related to sensitivity to TCA, we also determined absolute thresholds (AT) for TCA in wine. Best estimate thresholds for the CRT and AT were 6.3 and 2.8 ppt, respectively. CRT and AT were also significantly positively correlated ($r = 0.43$). Few of the Ss were "serious" wine drinkers as assessed by a wine knowledge test. Nevertheless, both CRT and AT were significantly negatively correlated with the score on a series of questions that assessed knowledge about TCA. These results provide a rational basis on which to assess the real impact of TCA in white wine and estimate what levels of TCA should be regarded as unacceptable. The results also provide evidence for the utility of this method for determining CRTs.

Extraction Dynamics of Seed and Skin Tannins during Maceration

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To manage tannin in red wine fermentations optimally, winemakers would like to know the following: when tannins are extracted during the alcoholic fermentation, from which part of the grape (seed or skin) the tannins are derived, and from where in the tank the extraction occurs (that is, does seed tannin extraction at the bottom of the fermentor contribute significantly to the tannin in red wine). The purpose of this investigation was to better understand the tannin extraction process from grapes into must/wine during maceration. The experiment was conducted with Pinot noir fruit in commercial-scale open-top fermentors and with cap management by punch down. Wine samples were taken daily during the alcoholic fermentation until dry and at three different depths in the tank: bottom, middle, and top (in the cap). For each sample, the concentration of skin and seed tannin was determined. At the peak of alcoholic fermentation, a large amount of skin tannin was released from the cap, with an increasing amount of seed tannin extracted with time. At the end of fermentation, 50% of tannin present in the wine was derived from the seed, all of which was essentially extracted from the cap. Based upon samples collected from the bottom of the fermentor, little if any of the tannin in the wine was derived from seeds lying at the bottom. In parallel, Pinot noir grapes harvested in the same block were managed by pump-over instead of punch down. The tannin concentration determined in the final wine was lower with a higher proportion of skin tannin.

Identification of New Volatile Compound Markers of American Oak Wood (*Quercus alba*)

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Distinguishing between the oak wood of European (*Quercus robur* L., *Quercus petraea* Liebl) and American species (*Quercus alba*) used in cooperage is commonly based on the chemical analysis of extractive compounds. Compounds that have been used as markers to determine the origin or species of oak wood include scopoletin, total β-methyl-γ-octalactone and *cis*-/*trans*-β-methyl-γ-octalactone ratio. Significant differences in the concentration of these markers allows one to distinguish between European and American oak wood. In this study, new markers of American oak wood, namely 3,4,5-trimethoxybenzaldehyde and 3,4,5-trimethoxybenzylalcohol, were identified for the first time from the statistical treatment of GC-MS data of a total of 720 European and American oak wood samples. ChromStat and MasStat software was used to analyze the volatile compound data. The presence of these substances was proved by MS-spectra and co-chromatography. European and American oak wood can be distinguished by the higher levels of

3,4,5-trimethoxybenzaldehyde and 3,4,5-trimethoxybenzylalcohol found in samples of American oak wood. Concentrations of both these compounds are approximately 6 to 10 times higher in American than European oak wood. These compounds are identified and quantified for the first time in oak wood and could be considered as markers of the botanical species *Quercus alba*. A possible metabolic pathway for these substances in wood is suggested. The difference between American and European oak wood may be explained by differences in the metabolism relating to the intermediary cinnamic product and influenced by methyltransferase.

Volatile Sulfur Analysis of Wines by Headspace Solid-Phase Microextraction/Gas Chromatography-Pulsed Flame Photometric Detector Technique

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Volatile sulfurs are a group of chemical compounds associated with onion and cabbage odors, which often cause off-flavor in wine. In 2000, approximately one-fifth of all California wines developed an unpleasant odor due to the formation of hydrogen sulfide. For decades, wineries have had recurring problems with the development of off-flavor attributed to the high sulfide levels in their wine. In this study, a rapid and sensitive headspace solid-phase microextraction (SPME)-gas chromatography-pulsed flame photometric detector (PFPD) was used to study the volatile sulfur compounds in wine. In a tightly capped 40-mL vial, 15 mL of wine sample was equilibrated for 10 min at 30°C in a water bath. A Carboxen/PDMS fiber was exposed to the headspace for 15 min to extract sulfur compounds. Following extraction, the fiber was inserted into injection port of GC system for thermal desorption at 300°C for 5 min. The headspace extracts were analyzed with gas chromatography equipped with a PFPD in the sulfur mode. This method can effectively analyze hydrogen sulfide, ethanethiol, methanethiol, dimethyl disulfide, diethyl disulfide, methional, and methionol in wines. Their concentrations in wines from approximately 12 cooperating commercial wineries were determined.

Influence of German Oak Chips on Red Wine

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Maturing wines in small oak barrels facilitates an accelerated polymerization of wine tannins due to the impact of oxygen. The concurrent flavoring of the wine can be also achieved by adding oak chips to wines stored in stainless steel tanks or neutral barrels. Although addition of oak chips is a common practice in many countries, it is forbidden in the European Union. German red wines were aged either in small oak barrels or in the presence of oak chips. Species and geographical origin of the oak material, degree of toasting, size, and time of addition were varied. The wines were assessed by 12 judges in randomized order and in duplicate by descriptive analysis. Increased toasting enhanced spicy, roasted, and dried fruit attributes, but the strongest toasting reduced flavor intensities. For medium toast, *Quercus robur* species revealed a stronger flavor potential than *Quercus rubra* species, enhancing spicy, dried plum, coffee, and vanilla/caramel characters. An aroma-extract-dilution-analysis (AEDA) of oak chips extracted by model solutions revealed 4-vinylguaiacol, syringol, iso-eugenol, vanillin, furfural, *cis*-oak lactone, and guaiacol as the most potent aroma compounds. A good discrimination between both enological treatments were achieved by electronic nose analysis. While ranking data of wine experts showed a clear preference for wines treated with oak chips, a small consumer experiment revealed no sensory preference for oak chips, regardless if the consumer was aware of the treatment. The major indicator of preference was the overall liking for oaked red wines, regardless of the source of the aroma.

Regulation of Anthocyanin Biosynthesis in *Vitis vinifera* Cell Culture Following Treatment with Sucrose, Jasmonic Acid, and Continuous White Light Irradiation

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Anthocyanins are a class of flavonoid pigment of particular importance in grapes and red wine. Their synthesis in grape berries occurs as part of the ripening process, with transcriptional regulation of UDP-glucose:flavonoid glucosyltransferase (UFGT) the major pathway control point. It is unclear whether similar regulatory mechanisms apply for a *Vitis vinifera* cell culture system where the aim is to synthesize high levels of anthocyanins. There have been limited studies of transcriptional regulation in *V. vinifera* cell culture, and it is anticipated that the absence of developmental cues in undifferentiated tissue could impact on anthocyanin pathway regulation. Further understanding of regulatory events is required to facilitate rational bioprocess optimization, which has been suggested as a conceptual framework to overcome plant cell culture limitations. Conditions known to improve anthocyanin production—sucrose (S), jasmonic acid (JA), and light irradiation (L)—were used to probe the pathway and gene expression data generated using real-time quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR). The UFGT gene, phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), dihydroflavonol 4-reductase (DFR), and flavonoid 3'5' hydroxylase (F3'5'H) were assayed, with the data normalized against that of β-tubulin. Additive enhancement of anthocyanin biosynthesis in response to S+JA+L involved up-regulation of all biosynthetic genes by S+JA, while maximal UFGT induction under these conditions required light irradiation. This was despite minimal transcriptional regulation by light irradiation alone. Sucrose and jasmonic acid individually generated very different patterns of transcript abundance; thus, the additive enhancements in anthocyanin synthesis appeared to be mediated by different but complementary mechanisms.