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AMERICAN SOCIETY FOR ENOLOGY AND VITICULTURE

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Abstracts are those submitted and accepted through the 2009 Call for Abstracts only.

Wine Aroma and Mouthfeel

Bound and Unbound Tannin Extraction from Fruit during Fermentation of Merlot Grapes

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Tannin content plays an important role in the quality of red wines. The objective of this research was to completely account for tannin extraction from the fruit to the finished wine throughout fermentation. This study addresses extraction and retention of tannins and polymeric pigments and provides additional quantitative information on tannin partitioning during the extraction process. Merlot grapes from 101-14 rootstock and full irrigation were harvested at the Oakville Experimental station, and were fermented in triplicate of 5-gal lots at the UC Davis Winery. The use of a controlled, small-scale fermentation was to account for all of the tannin in fruit at harvest and to show how it is partitioned during fermentation, taking into account tannin that might be covalently bound to cell wall material in the pomace and gross lees. Previous work demonstrated that berry cell walls bind tannin and play a role in tannin extraction. A procedure referred to as the acid butanol/ferrous sulfate method was used in an attempt to detect tightly bound tannin in the insoluble material from the skin, mesocarp, pomace, and lees of the small-scale fermentations. This method enables the amount of bound tannin in berry components along with the tannin that is usually extracted with acetone to be assessed. The results demonstrate that bound tannin was low in skin, very low in mesocarp, higher in pomace, and highest in lees. These are the first results that can completely account for 100% of tannin extraction from fruit during fermentation.

Funding support: American Vineyard Foundation.

Volatile Profiling of Cabernet Sauvignon Wine Using Comprehensive Two-Dimensional Gas Chromatography

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The fields of separation science and sensory science have advanced our knowledge of how grape and fermentation-derived compounds contribute to wine aroma. It is now apparent that wine aroma is dependent not on any one particular compound but on the combination and ratios of odor active compounds present. The application of new analytical techniques, namely comprehensive two-dimensional gas chromatography (GCxGC), allows the investigator to measure a range of compounds in a single analysis rather than specific compounds in isolation. This study presents the optimization and application of a HS-SPME method for analysis of trace wine volatiles by GCxGC-TOFMS. The objective of this research was to develop a sensitive and reproducible analytical technique that was capable of characterizing the wine headspace volatile profile of commercial and research Cabernet Sauvignon wines. The methodology allowed for the simultaneous analysis of over 400 different analytes including potent

*indicates corresponding author

Wine Aroma and Mouthfeel – CONTINUED

aroma compound classes such as monoterpenes, norisoprenoids, sesquiterpenes, and alkyl-methoxypyrazines, which have been previously documented to contribute to wine aroma. The improved sensitivity and separation efficiency of the technique also allowed for the identification of a number of volatile compounds not previously reported in Cabernet Sauvignon wine. Application of multivariate statistical techniques including two-dimensional hierarchal clustering and PCA provided information about which of these compounds may contribute to vintage and regional aroma characteristics in Cabernet Sauvignon wines from Australia.

Characterization of the Aromatic Profile of Cabernet Sauvignon Grapes and Wines from the Central Coast at Different Stages of Maturation

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Although the timing of harvest is widely considered to be of critical importance with respect to wine quality, little information is available on chemical and aroma profiles of winegrapes during maturation. Therefore, this study was conducted to provide winemakers with useful information for determining the "ideal" stage of maturation, which may differ according to the specific style of wine they pursue. Cabernet Sauvignon grapes from Paso Robles (Central Coast, California) were harvested at six different stages of maturation, between 20 and 30 Brix, over three vintages (2006–2008). Berries were analyzed for sugar concentration, TA, polyphenols, and aroma profile. The aroma profile identified 52 volatile compounds by head-space SPME-GC-MS. Berries sampled at early Brix were characterized mainly by C6-aldehydes and alcohols, known to impart green aromas to the berries. C_{13} nor-isoprenoids and terpenes reached their maximal concentrations in berries sampled 40 to 60 days after veraison (24–26 Brix). These compounds are characterized by fruity and floral aromas. Grapes from later harvests showed high concentrations in C7–C10 alcohols, aldehydes, and ketones. The odors of these compounds are typically not related to ripeness of fruit. Results show that the developmental pattern during fruit maturation varies among volatile compounds and suggest that monitoring the changes of C_{13} nor-isoprenoids and terpenes during maturation could be used to assess fruit maturity.

Wine Aroma and Mouthfeel – CONTINUED

Impact of Terroir, Vintage, and Winemaking on the Aromachemical Composition of German Riesling Wines

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Terroir, the specific combination of a vineyard's soil composition, mesoclimate, and topography in interaction with the vines, has been shown to significantly influence the sensory properties of wines. With volatile compounds driving sensory perception, this study assessed the impact of terroir on the aromachemical composition of German Riesling wines, together with the influence of vintage and winemaking. For this, 12 highly diverse vineyard sites from 10 wine estates were selected in the Pfalz region, covering sites on limestone, sandstone, greywacke, basalt, and breccia from the Rotliegend age (Lower Permian). One year later, the research was extended to 13 additional sites in the growing regions Mosel/Saar, Ahr, Nahe, and Rheinhessen, comprising further sites on limestone and Rotliegend material and sites on slate and porphyry. Extensive soil analyses were conducted for the Pfalz vineyards, and meteorological data were collected from weather stations installed nearby or on site. For the 2004 and 2005 vintages, one portion of grapes from each site was subjected to a standardized winemaking protocol, while the major portion underwent customary vinification at the participating wine estates. Forty-six volatile compounds were identified using headspace-SPME. Analysis of variance revealed systematic aromachemical differences among the wines from the different geological substrates, for both vintages. Vintage and vinification also proved to be a significant source of variance. Discriminant analysis showed a clear grouping of the samples by substrate type. Numerous correlations between pedo-meteorological parameters and volatile compounds were revealed by partial least squares regression. These results clearly indicate that terroir has an impact on the aromachemical composition of wines.

Electronic Nose Analysis on the Effect of Prefermentation Cold Soak of *Vitis vinifera* L. cv. Cabernet Sauvignon Grape and Wine Volatiles

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Cold soak is a prefermentation maceration process at cold temperatures for a defined time to enhance red wine color. Cold soak may also alter aroma and flavor compounds of red wines. This study focused on monitoring aroma changes during a five-day cold soak period and determining differences in aroma volatiles of *Vitis vinifera* L. cv. Cabernet Sauvignon wines. Cabernet Sauvignon grapes in the 2007 and 2008 growing seasons were harvested and designated either to control or five-day cold soak treatment. A commercial conducting polymer electronic nose (ENose) was used to monitor possible volatile changes throughout cold soak and following fermentation. Primary juice and wine chemistries, SPME GC-MS analysis, and sensory evaluation were used for comparison to ENose data. ENose results found significant differences in the aroma component of juices during cold soak and resulting wines of both seasons. GC-MS results showed variation in individual volatile compounds. Sensory evaluation showed varying results dependent on vintage year.

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Wine Aroma and Mouthfeel – CONTINUED

Effect of Grape Skin Contact on Cabernet Sauvignon Wine Aroma Compounds

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Small-scale fermentations were conducted to investigate the effect of grape skin contact times on the development of aroma compounds in Cabernet Sauvignon wine. Four fermentation treatments were monitored: (1) skins fermented with juice for the duration of fermentation; (2) skins fermented for 72 hr, then removed from fermentation; (3) juice fermented without skins; and (4) skins fermented in a model juice solution. Additionally, skins were allowed to steep in a model wine solution, with no yeast inoculation. Headspace solid-phase microextraction (HS-SPME) was used to extract volatile compounds from wine fermentations throughout the course of fermentation. Gas chromatography-mass spectroscopy (GC-MS) was used to identify and quantify volatile compounds. Significant differences ($p < 0.05$) were found among skin contact treatments for levels of yeast-derived ethyl esters, as well as phenylethyl acetate, 1-hexanol, 2-phenyl ethanol, and β-damascenone.

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Disease Resistance

Quantitative Genetics of Root-Knot Nematode Resistance in a *Vitis* Rootstock Hybrid Population

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Root-knot nematodes, *Meloidogyne* spp., are a major pest of grapes worldwide. In order to determine the value of rootstocks as parents in breeding improved nematode-resistant rootstocks, a Design II mating array was developed with four female parents and six male parents. The female parents were the rootstocks Dog Ridge, *Vitis rupestris* 187G, 106-8 Mgt, and 93-5 Couderc. The male parents were the rootstocks 216-3 Castel, 44-53 M, *V. rupestris* Constantia, Gravesac, 3309 C, and 3306 C. Each female parent was crossed to each male parent to generate 24 seedling populations. The experiment was organized as a randomized complete block design, with 10 seedlings per seedling population in each of six replicates. Seedlings were inoculated with 1500 infectious J2 root-knot nematodes once the seedlings had two true leaves. Seedling roots were stained in an eosin-Y solution six to seven weeks after inoculation. Each root system was visually examined to observe and quantify egg masses. The mean number of egg masses per seedling population, per female parent, and per male parent demonstrates differences among female parents, male parents, and seedling populations. This information is useful in identifying candidate parents for rootstock breeding, as it describes the expected transmission of nematode resistance to the progeny of the parents sampled in this study. Among the female parents, Dog Ridge transmits the highest level of nematode resistance to its progeny, while *V. rupestris* 187G and 93-5 Couderc transmit low levels of nematode resistance to their progeny.

Bot Canker Disease of Grapevines in California: From Identification to Control

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Grapevine canker diseases are some of the primary factors limiting vineyard longevity and productivity as well as increasing management costs. Grapevine cankers cause dieback and death of spurs, arms, cordons, and trunks, and eventual vine death. Fungal species in the family Botryosphaeriaceae have been recently recognized as the most common fungi isolated from grapevine cankers in California. Morphological identification along with multigen phylogenetic analysis showed that at least nine Botryosphaeriaceae spp. occur on grapevines in the state. Pathogenicity studies showed all nine Botryosphaeriaceae spp. able to infect both young and mature tissues as well as green shoots of the new vegetative growth, causing cankers, vascular discoloration, and/or otherwise dark streaking of the wood, and thus, concluding their importance as grapevine pathogens in California. In addition, field surveys conducted in other grapegrowing regions in

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Disease Resistance – CONTINUED

the United States and Mexico have also shown the important role of these fungi in grapevine health. Botryosphaeriaceae spp. are wood pathogens infecting mainly through pruning wounds. Therefore, knowledge of low-risk infection periods and pruning wound susceptibility are critical in deciding appropriate timing for pruning and wound treatment. Epidemiological studies throughout the state have revealed that Botryosphaeriaceae spores are mainly trapped following rainfall from November to March under California weather conditions. Duration of susceptibility of pruning wounds to infection by Botryosphaeriaceae spp. was also studied. Furthermore, this study has also evaluated the efficacy of double pruning and chemical products to reduce infections caused by Botryosphaeriaceae.

Improved Methods for Killing Old Grape Roots

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Soil fumigation can result in complete kill of old grape roots to depths of 1 to 2 m, depending on fumigant, while also providing 99.99% nematode control. Soil fumigation serves the purpose of reducing soil-borne pests, killing grapevine root systems, and stimulating growth of replanted vineyards. We are searching for alternatives to fumigation. Applications of glyphosate or triclopyr herbicides applied to leaves, trunks, or shoots of various wine and table grapes from July through mid-November provide root death, but not below 0.5-m soil depth. Reirrigation of old grape stumps 18 months after treatment indicated 98% survival of own-rooted Ruby Seedless vines. Surviving vines flourished into full production three years after foliar-applied herbicide treatments. February and March applications of glyphosate plus MorAct adjuvant painted to recently cut trunks of Pinot noir, Freedom, or 420A root systems resulted in 4% vine survival at 18 months after reirrigation was begun. Wintertime treatments to grape stumps currently provide our most successful root-killing treatment beyond use of soil fumigants. Three years after wintertime treatments, roots of Thompson Seedless and Ruby Seedless were decimated and notably missing from the surface 2 m of soil profile. The field surface must be maintained as weed-free during the one year or longer waiting period to reduce prevailing nematode populations. The 8 years required for normal death of old grape roots can be reduced, thus providing an indirect approach to starving old soil ecosystems, a component of the grape replant problem.

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Disease Resistance – CONTINUED

Biology and Management of Japanese Beetles in Vineyards

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The Japanese beetle, *Popillia japonica*, an invasive pest first found in North America in 1916 and now established throughout most of the eastern United States, continues to expand its range into the Great Plains and southcentral states. It feeds on about 300 wild and cultivated plants in 79 familie and grapes (*Vitis* spp.) are among its favored hosts. Six economically important grape cultivars were selected to evaluate the impact of Japanese beetle defoliation on vine growth, primary bud cold hardiness, yield, and berry composition from vineyard establishment through production. Data indicate that while defoliation can reduce cordon growth, winter hardiness, and yield of susceptible cultivars, growers can reduce frequency of cover sprays in newly planted vineyards by half and still receive the same benefits of complete management. There were no significant effects of defoliation on total soluble solids, juice pH, or titratable acidity at harvest. Adult Japanese beetles are mainly leaf feeders, but also exploit ripening berries. In the clusters, Japanese beetles facilitate aggregation and injury by the obligate fruit-feeding native green June beetle, *Cotinis nitida*, through physical wounding and eliciting yeast-induced fermentation volatiles the latter species exploits in host-finding. Field traps baited with combinations of grapes and beetles demonstrate that fruits injured by Japanese beetle alone become highly attractive to green June beetles because of the high amount of fermentation compounds emitted compared to intact grapes. Establishment and spread of the Japanese beetle throughout viticulture regions of the United States is likely to increase its status as a vineyard pest.

Funding support: New Crop Opportunities Center, University of Kentucky (USDA Special Grant) and USDA-Southern Region Sustainable Agriculture Research and Education Grant.

Breeding Grapes with Resistance to Powdery Mildew (*Erysiphe necator*)

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We have been using molecular breeding tools to introgress powdery mildew (PM) resistance from a variety of *Vitis* sources into *Vitis vinifera*-based cultivars. Resistance is available from a number of species and we are pursuing those with the potential for marker assisted selection (MAS). Progress in developing and using MAS and in developing resistance has been made on many fronts. Several accessions of *Muscadinia rotundifolia* and their proven genetic markers have been used to evaluate parents and progeny from our crosses. We verified the single dominant gene (locus) nature of resistance from a *V. vinifera* table grape, Kishmish vatkana, under California environmental conditions. The above-mentioned sources were used to make crosses combining resistance from *rotundifolia* and *vinifera* selections. Powdery mildew resistance from

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Disease Resistance – CONTINUED

V. cinerea was used in crosses, but few progeny were resistant and resistance expression was moderate. Resistance from wild Chinese species, previously noted at the USDA-Parlier, was confirmed and used in crosses. We are searching for alternative sources of resistance in Middle Eastern table grapes related to Kishmish vatkana. The knowledge and results gained from this work will lead to the development of wine and table grape selections with multiple PM resistance genes. The integration and complexing of multiple sources of resistance will ensure that resistance is broad and durable and that it results in low input, environmentally "green" grapevines.

Funding support: American Vineyard Foundation and Louis P. Martini Endowed Chair.

Breeding Winegrapes with Resistance to Pierce's Disease

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Strong progress in breeding Pierce's disease-(PD) resistant winegrapes has been made using a form of *Vitis arizonica,* b43-17, from Monterrey, Mexico. Previous efforts produced a genetic linkage map, which located a major locus, *PdR1*, controlling resistance to *Xylella fastidiosa*, the bacterial agent of PD. Simple sequence repeat (SSR) markers tightly linked to *PdR1* are being used to screen seedling populations and have, in conjunction with intensive vine-training efforts, reduced the seed to seed breeding cycle to three years. Seedling populations from our 2007 crosses were screened for markers linked to *PdR1* and in some cases they were screened for markers linked to the powdery mildew resistance locus, *Run1*. Only those seedlings with the markers were planted in the field. We have made wines for two seasons from PD-resistant selections that are 87% *V. vinifera* and contain *PdR1*. They rated very favorably with wines from Cabernet Sauvignon and Pinot noir grown in the University vineyards and made using the same small small-scale fermentation techniques. In 2009, we will make crosses to produce *PdR1* containing progeny with ≈97% *V. vinifera* from crosses with many high-quality *V. vinifera* winegrapes. We will also produce small-scale wines from replicated vines that are ≈94% *V. vinifera*, have tested PD-resistant, and have *PdR1*.

Funding support: CDFA Pierce's Disease Board, American Vineyard Foundation, and Louis P. Martini Endowed Chair.

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Wine Aging

Free Radicals in Oxidizing Wine and Factors That Influence Their Formation

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Oxidation reactions in wine lead to a host of chemical changes, of which many affect perceived quality. Recent studies have demonstrated that free radicals are important intermediates in wine oxidation, yet the identity and fate of these radicals have not been established. In this study, electron paramagnetic resonance spectroscopy was used to detect and identify several free radical species in a wine system under oxidative conditions with the aid of spin traps. The 1-hydroxyethyl radical dominated, but the hydroxyl radical was also observed, while the hydroperoxyl radical was elusive. Also, the addition of iron and/or copper to a red wine resulted in a marked increase in observed spin adducts, demonstrating that trace levels of metals are essential catalysts in the oxidation of wine. The addition of catechin to a white wine containing excess sulfur dioxide had no effect on the initial rate of radical formation, but was prooxidative in the later stages of the experiment. Finally, sulfur dioxide was shown to inhibit radical formation in a concentration-dependent manner.

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

Port Wine Oxidation Management: A ChemoInformatics Approach

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Chemical changes that occur during winemaking resulting from both enzymatic and nonenzymatic reactions are responsible for differences in sensorial perception throughout the process, aging included. The chemical parameters that affect the extent and rate of each reaction can be calculated by theoretical modelling, and ultimately, if the overall "topography" of the system created by the contribution of the kinetic parameters of each mechanism is known, the overall process can be controlled. Kinetic studies are important in establishing temporal relationships among Port constituents and can be used to infer the chemical network of reactions—the "chemiomics." Although previous studies have shown that 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) may be formed by several pathways, it is not known how oxygen regimens and storage temperature contribute to its formation. This problem has an important relevance for Port wine management of both vintage (bottles) and *colheita* (barrel) stock where the correct temperature and O_2 permeability must be controlled in order to attain the desired sensory quality of the aged wine. A Port wine database including other compounds formed by important flavor mechanisms (such as Maillard) was constructed. The goal was to

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Wine Aging – CONTINUED

define the characteristic feature space and thereafter classify samples during storage. This tool would be useful in oxidation management of Port by comparing the quality of the sample with the quality of stock wine. The predictive model can be used to optimize wine aging through storage temperature and oxidation regimens with the aim of producing quality-specific chemical profiles.

Effect of Different Microoxygenation Treatments on the Evolution of Cabernet Sauvignon Wine Composition

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The effect of microoxygenation on the composition of red wine was investigated where oxygen was applied at both low and high rates (5 and 20 mL/L/month) together with oxygen permeated through polyethylene tanks. A control with no oxygen was also included. The study was performed in triplicate for each treatment using commercially made Cabernet Sauvignon wine stored in 9 x 300 L stainless steel and 3 x 300 L Flextanks at 16°C over 16 weeks. Spontaneous malolactic fermentation occurred simultaneously with microoxygenation treatments. Results indicated that color parameters, including pigments resistant to SO_2 bleaching and the wine colour density, were the most affected by microoxygenation. No firm trend in the development of the total tannins and individual phenolic compounds was observed. However, there was an initial increase in the pigments resistant to SO_2 bleaching for all treatments, followed by a decrease in these pigments and in color density, with the rise and subsequent decline most marked in the higher oxygenation treatments, suggesting a complex interplay of polyphenol oxidation processes. Wine in the polyethylene Flextanks showed a consistent increase in the pigments resistant to SO_2 with levels at most data points significantly higher ($p < 0.05$) than those of the control. Future work includes analyses of aroma compounds to evaluate the overall effect of the microoxygenation treatments.

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Wine Aging – CONTINUED

Effects of Aging on the Composition and Physicochemical Properties of Red Wine Tannin Fractions

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A new SPE method to isolate polymeric polyphenols from red wines, based on a copolymer Oasis HLB cartridge, has been developed and validated. The method affords a wash fraction (F1) containing the majority of the nonpolymeric material present in a red wine and yields the polymeric species as two distinct fractions (F2 and F3) which possess different physicochemical properties. There appears to be a relationship between the amount of material found in each polymeric fraction and wine age. The differences observed between the two polymeric fractions obtained from red wines using this method have been further investigated in terms of the structural characterization and the effects that aging has on the two fractions. F2 and F3 were isolated from selected years within a 50-year vertical series of Cabernet Sauvignon wines. The difference in hydrophobicity between the fractions was measured and the hydrophobicity was shown to generally increase with age. Somers color measurements were also performed and, with age, the proportion of ionizable color and nonbleachable pigments of F2 relative to F3 diverged. F2 contained less ionizable color, nonbleachable pigments, and total phenolics than F3. F2 and F3 from a 30-year vertical series of Shiraz and Cabernet Sauvignon were also isolated and showed similar trends, with some differences observed for the behavior of the tannin fractions from Shiraz, which generally were more similar in properties. These findings have implications for the oxidation and development of red wine tannin during aging.

Funding support: Grape and Wine Research and Development Corporation, with matching funds from the Australian Government.

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Vine Nutrition and Grape Composition

Sugar Accumulation Disorder May Be Able to Spread from Affected Tissue to Healthy Tissue

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Sugar accumulation disorder (SAD, formerly known as berry shrivel) is a ripening disorder of unknown cause that occurs in the North Coast of California. Afflicted fruit has low Brix, poor anthocyanin accumulation in red varieties, low pH, and a shriveled, puckered appearance. SAD fruit is usually removed from the vineyard before harvest. In 2003, duplicate chip buds were taken from five vines that historically exhibited SAD symptoms (SAD wood sources) and from five vines that did not exhibit such symptoms (healthy wood sources) and budded onto healthy rootstock at the UC Davis Oakville Experimental Vineyard. Vines propagated from SAD sources had fruit of lower Brix than vines propagated from healthy wood sources. This trend has been true since 2006, the first year these vines had fruit. In 2007, chip-budding experiments were carried out on these vines and other vines in the same block. The limited fruit available in the 2008 season indicated that when SAD scion material was grafted onto a healthy vine, the Brix of the fruit from the SAD bud was similar to the Brix from the native fruit on the vine, and lower than fruit from an ungrafted vine. Conversely, when healthy buds were grafted onto SAD vines, the Brix of the fruit from the healthy buds was similar to the native SAD fruit from these vines. These preliminary data suggest that whatever causes SAD can spread to healthy tissue. This suggests a pathogenic cause of the disorder at this site, although the vines have tested negative for all known grapevine pathogens.

Managing Vineyard Cover Crop to Influence Vine Vigor and Fruit Quality of Pinot noir in Oregon's Willamette Valley

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The Willamette Valley of Oregon receives ~1000 mm of rain per year, from October to June, resulting in high soil moisture from budbreak to bloom. This is followed by a dry summer from fruit set to ripening. To best manage vine vigor early while conserving soil moisture later in the season, a cover-crop study was conducted using alleyway management regimes: clean cultivated, alternate row tillage and solid cover of an established perennial grass cover crop. The study was conducted in two commercial Pinot noir vineyards in the Willamette Valley during 2007–2008. Vegetative growth was not significantly impacted by alleyway management in year one of the study; however, by the second year, vines had reduced canopy growth and pruning weights with the solid sod cover. Although there was a reduction in vine canopy density and vine size, as evidenced by sunfleck analysis and pruning weights, water stress was not reached in the vines with any treatment during the first or second season of the study. Cover-crop management method did not significantly impact soil moisture within the vine row. Fruit maturity,

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berry weight, and yield were not affected by cover-crop management treatments. However, harvested fruit differed in concentrations of anthocyanins, total phenolics, and yeast assimilable nitrogen with alleyway management. Yeast assimilable nitrogen concentration was lower in solid sod cover treatments and was below the sufficiency level for healthy fermentations while other treatments were within sufficient levels. However, the solid cover treatment resulted in higher anthocyanins and total phenolics.

Funding support: Oregon Wine Board.

Effect of Ripeness Level and Variability on Fruit and Wine Composition of Pinot noir

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The flowering process in grapevines spreads over two seasons and the interactions among genotype, environment, and management practices give rise to considerable variability, resulting in asynchronous development of individual flowers. This variability will then be reflected in the resulting population of berries used for winemaking. Although uniformity or synchrony of development is perceived as advantageous, there is no experimental evidence linking variability in ripeness with wine composition and sensory properties. Pinot noir fruit was harvested at an average of 25 Brix, was hand destemmed, and individual berries were segregated into five discrete levels of soluble solids ranging from <23 Brix to >26.5 Brix using sucrose solutions. Histograms of the original population distribution were used to reconstruct the "control" fruit. Berry samples from each ripeness level and control were used to characterize berry architecture and composition. The fruit groups were fermented in three replicates. Seed number was less abundant and berry size was smaller the higher the ripeness level attained, indicating that conditions during ovule fertilization play a role in the berry sugar metabolism. Seed number and fresh weight per berry showed the highest variability (cv = 20%) and explained 94% of the variation in berry weight. Wine total anthocyanins and phenols increased with the level of ripeness while malate and tartarate decreased. All wines completed alcohol and malolactic fermentations except the ripest level (>26.5 Brix), where malate was not metabolized and residual sugar reached 16 g/L.

Funding support: New Zealand FRST grant CO6X0707.

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Vine Nutrition and Grape Composition – CONTINUED

Influence of Soil Properties and Fertilization on Leaf and Petiole Nutrient Levels in a Paso Robles, California Cabernet Vineyard

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Soil physical and chemical properties affect vine nutrition, as indicated by leaf and petiole nutrient content, in a way that may directly impact wine properties. There is renewed interest in the effect of soil nutrients, as influenced by soil type and soil mineralogy, on fruit and wine characteristics. This study examined leaf and petiole nutrient levels and fruit and juice characteristics of own-rooted Cabernet Sauvignon vines grown on four distinct soil types in the same Paso Robles vineyard. The soils were classified as Palexeralfs, Haploxeralfs, Haploxerolls, and Haploxererts and exhibited important morphological differences in color, coarse fragment content, texture, water-holding capacity, and hydraulic conductivity. The soils also showed important differences in chemical characteristics and nutrient availability. The soils covered contiguous vineyard patches planted with the same cultivar, on its own roots. The vineyard was irrigated and fertilized. Mesoclimatic conditions and slope aspect were similar. Soils were analyzed for physical and chemical differences to determine the influence of the four contrasting soil types on differences in vine growth, water stress, and plant nutrient levels. Differences in cation exchange capacity and cationic balance in the soil solution appeared to affect nutrient availability to the vines and likely contributed to the observed differences in the plant and fruit characteristics. Berries harvested on the four blocks exhibited different sensory attributes, as determined by a tasting panel.

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Wine Microbiology

Global Profiling of the Bacterial and Yeast Ecology in Wine Production

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Winemakers require rapid and comprehensive monitoring of microbes at all stages in wine production. Since culture-dependent methods for microbial identification are laborious and time-consuming, numerous culture-independent assays have been developed. These include methods, such as real-time PCR, which are now routinely used by wineries and wine service laboratories to enumerate specific microbial species or genera. However, global profiling techniques—such as PCR-denaturing gradient gel electrophoresis—which identify all the microbial contents in a single analysis have found little commercial utility, mostly because they are technically problematic and low throughput. To surmount this we adapted terminal restriction fragment length polymorphism (T-RFLP) analysis for high throughput determination of the microbial composition of musts and wines. We generated T-RFLP databases of wine-related yeast and bacteria and tested them empirically on numerous wine-related yeast and bacteria to validate the predicted terminal restriction fragment lengths and assess strain-to-strain variability. T-RFLP was then used to profile numerous finished wines and fermentation samples. Our study suggests that T-RFLP analysis is a relatively simple tool for high throughput determination of the dominant microbial diversity present in wine production. In addition, by providing a global profile, T-RFLP nicely complements targeted microbial enumeration by real-time PCR analyses.

Selection of Wine Yeast for Making White Wine with a High Level of Aromas from Koshu Grapes

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Koshu is a traditional, Japan-specific *Vitis vinifera* grape, and the wine is gaining attention because of its good pairing with Japanese foods. However, Koshu wine has relatively high levels of phenolics and a relatively flat taste. At times, Koshu wine exhibits phenolic off-flavors because of the phenolics. We had developed the Koshu wine made by the sur lees method, and could overcome the flatness. This time, we challenged another style of Koshu wine by screening wine yeasts without producing phenolic off-flavors from commercially available yeast strains mainly provided by Sceti Company, Japan, and Lallemand. Of 48 strains tested, eight strains did not produce both 4-vinyl phenol and 4-vinyl guaiacol by high-sensitive HPLC with a fluorescent detector. Of eight strains, one strain (LYCC52 DGI 288) gave a Koshu wine with high levels of aromas such as isoamyl acetate, ethy hexanoate, hexyl acetate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate, and 2-phenylethyl acetate, and levels were much higher than in wines made with EC1118 or W3 strains. The flavor was determined as similar to Sauvignon blanc. The strain 228 produced a quality wine by fermenting

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at a temperature below 18°C. The aromas were analyzed by GC-MS with 1000-fold condensation, but 3-mercaptohexanol and S-containing aromas were not found. The Sauvignon blanc-like flavor might be derived from the high levels of various ethyl esters and hexanol because of its greenish aroma.

Impact of *Saccharomyces* and Non-*Saccharomyces* Yeast on the Flavor and Aroma of Pinot noir

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Yeast present on grapes or winery equipment and yeast deliberately inoculated into must play an important role in the development of wine flavor and aroma. However, it is not well understood what impact the growth of a specific yeast strain has on a wine's sensory profile, especially relative to cultivar-specific aromas. Previous research investigating the impact of yeast on red wine production has relied on the inoculation of a large population of a selected yeast strain. However, this makes it difficult to draw any conclusions on the contribution of individual yeast strain, as indigenous yeast and bacteria present on the grapes have been shown to contribute to the final aroma and flavor of a wine. Although a technique such as heat could be used to kill microorganisms present on the grapes, it may result in changes in grape flavor compounds and/or introduce new flavor components. In order to overcome this problem, the use of high hydrostatic pressure (HHP) to eliminate yeast and bacteria from grapes prior to inoculation was investigated. HHP has been shown to have minimal impact on flavor and aroma compounds when applied in other food systems. Initial experiments were performed to determine the ability of HHP to kill common wine microorganisms such as *Saccharomyces cerevisiae*, *Acetobacter*, *Brettanomyces*, and *Oenococcus oeni* in grape must. Results showed that HHP eliminated all of the microorganisms to below detectable limits. Microscale red wine fermentation vessels that could be sterilized by autoclave were constructed and used in conjunction with HHP. The influence of four *S. cerevisiae* strains and *Kluyveromyces thermotolerans* on Pinot noir was investigated by producing wines from HHP treated Pinot noir grapes. Sensory analysis of the wines using a trained panel was conducted.

Funding support: Oregon Wine Board.

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Analysis of Lipid Compositional Changes during Fermentation in Two Wine Yeast Strains with Varying Ethanol Tolerance

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Pertubations to the yeast biomembrane because of ethanol and other fermentation products have previously been implicated in stuck and sluggish fermentations, a pervasive problem in the wine industry that has significant economic consequences. Numerous studies have reported that the yeast *Saccharomyces* sp. undergoes variations in phospholipid composition because of increasing ethanol concentration during fermentation. However, it remains to be determined how critical the membrane composition is in completing a successful wine fermentation. Small-scale fermentations were carried out with two commercial wine yeast strains that previously exhibited markedly different sugar utilization and ethanol tolerance characteristics in defined, synthetic grape juice at high Brix levels. Polar lipids were extracted from yeast using a modified Folch method developed by our group. Identification of phospholipid classes were preliminarily determined using a novel liquid chromatography–mass spectrometer (LC-MS) method. Verification of phospholipid classes and structures were performed using tandem mass spectroscopy, data mining, and chemometric tools. Differences in membrane composition of the two strains were determined, together with how these differences may be related to ethanol tolerance. The LC-MS method developed is currently being applied to 30 yeast strains (from wine and other sources) at various time points during fermentation. The data will be used to elucidate any correlations between lipid composition and successful completion of fermentation—information that will be used subsequently to introduce greater ethanol tolerance into wine strains with other desirable flavor or quality attributes.

Funding support: USDA-CREES, American Vineyard Foundation, and California Competitive Grant Program for Research in Viticulture and Enology.

Kinetics and Genetics: 2,4,6-Trichloroanisole Production in *Streptomyces*

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Streptomyces, spore-forming, filamentous, Gram-positive bacteria, are ubiquitous in soil and other environments similar to those inhabited by fungi and, like fungi, produce musty odor compounds. Previously, *Streptomyces* wine cellar isolates were shown to O-methylate 2,4,6-trichlorophenol (TCP) to 2,4,6-trichloroanisole (TCA). In the current study, whole-cell cultures of additional *Streptomyces* strains were analyzed to determine how widespread TCA production may be. *Streptomyces coelicolor* A(3)2, *S. avermitilis*, and *S. griseus* subsp. griseus (sequenced) were also tested. Next, cell extracts prepared from *S. coelicolor* A(3)2 were used to characterize the O-methylation of TCP to TCA for

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a range of levels of substrates, cofactors, inhibitors, times, and temperatures. Formation of TCA was found to be extremely rapid and did not require induction by preexposure to TCP. Finally, a set of known and probable O-methyltransferase candidate genes in *S. coelicolor* was identified, amplified, cloned into a set of expression vectors, and tested for the ability to O-methylate TCP to produce TCA. Several of these genes have previously been shown to O-methylate other small phenolic compounds. Results from all three parts of this study provide new insights into conditions favoring TCA formation in *Streptomyces* and underscore the importance of preventing the establishment of patches of microbial growth in environments where food, wine, or consumer products are stored or shipped.

Effect of Alcoholic and Malolactic Fermentation Parameters on Acetaldehyde in Wine

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Acetaldehyde is the most important carbonyl compound formed during vinification and the major SO_2 binding factor in most wines. The main sources of acetaldehyde are yeast metabolism and the chemical oxidation of ethanol. A major reduction of acetaldehyde can be achieved by wine lactic acid bacteria (LAB) during malolactic fermentation. With the overall objective of reducing the levels of the wine preservative SO_2, the formation and degradation of the SO_2 binder acetaldehyde by yeast and wine LAB during pilot vinifications was studied. A preliminary survey of acetaldehyde levels in 125 commercial New York State wines revealed mean acetaldehyde concentrations of 43 mg/L and 24 mg/L for white and red wine, respectively, and the maximum level determined was 193 mg/L. The effect of several vinification parameters (yeast strain, grape variety, SO_2 and nutrient additions, temperature, and pH adjustments) on acetaldehyde formation and reuptake during alcoholic fermentation was investigated. Multifactorial analysis demonstrated a statistically significant correlation between SO_2 additions, fermentation temperature and grape variety and the production of acetaldehyde during alcoholic fermentation. Under the conditions chosen, no significant results were found for other vinification parameters. The acetaldehyde and malic acid degradation kinetics of 12 commercial wine LAB were investigated in wines of two different grape varieties. Overall, the degradation kinetics of malic acid and acetaldehyde were similar and acetaldehyde was depleted 0 to 10 days after the completion of malolactic fermentation. Results demonstrate that New York State wines show room for the reduction of acetaldehyde and hence, SO_2 levels, and that different vinification parameters can be adjusted to reduce final acetaldehyde levels.

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Rootstocks and Water

Evaluation of Rootstock Performance with Pinot noir on a High Magnesium Soil

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Relatively little information is available regarding the viticultural performance of common rootstocks on high magnesium soils. The goal of this project was to evaluate the performance of eight rootstocks with Pinot noir clone 667 across a range of soil exchangeable magnesium levels. Vines were planted in 1998 in a commercial vineyard located in the Edna Valley south of San Luis Obispo with the rootstocks 110 Richter (110R), 44-53 Malègue, Freedom, 1103 Paulson (1103P), 5 C Teleki (5C), St. George, 1616 Couderc (1616C), and Millardet et de Grasset 101-14 (101-14 Mgt). The soil exchangeable magnesium content ranged from 25% to >65% of the total soil cation exchange capacity. The planting was evaluated annually over four consecutive seasons from 2005 through 2008. Among the rootstocks, Freedom and St. George had consistently lower fruit yields. Freedom tended to have a lower average number of clusters per vine, while St. George had lower average cluster weights. 101-14 Mgt generally had the highest juice soluble solids at harvest, while Freedom generally had the lowest juice soluble solids. No significant differences were observed in berry weight, juice pH, titratable acidity, or juice potassium concentration among any of the rootstocks. Freedom, St. George, and 1103P generally had the highest winter pruning weights, while 44-53 Malègue and 1616C had the lowest. Significant differences were observed in bloom tissue nutrient contents, but yearly patterns were inconsistent. With increasing soil magnesium level, the berry weight tended to increase, while the cluster number, titratable acidity, and pruning weight tended to decrease.

Funding support: Pacific Vineyard Company.

Breeding for Salinity Tolerance in *Vitis*

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Soil salinity is becoming increasingly problematic in California vineyard soils and is accentuated by drought and the inability to leach salts from the rooting profile. As early as 1968, field studies reported as much as a five-fold difference in foliar chloride accumulation when *Vitis vinifera* cultivars are grafted onto non-*vinifera* rootstocks. The rootstock Ramsey (*V. champinii*) exhibited the most consistent NaCl tolerance in these trials. However, Ramsey often induces excessive vigor in the grafted scion and can lead to dense canopies, poor quality fruit, and reduced bud fruitfulness. Efficient breeding of grapevine rootstocks for both salt tolerance and scion devigoration requires a greenhouse-based assay capable of evaluating numerous test plants in a short period of time. Attempts to develop such a screen over the past 40 years have resulted in data that is comparable to field data, but for unknown reasons results in relatively small differences in chloride uptake; that is not significantly different between rootstocks and *V. vinifera*; or that is opposite to phenotypes observed in the field. We therefore developed and

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tested three separate assays to define the relative merit of a high input, low throughput system (hydroponics), a moderate input, moderate throughput system (greenhouse-based ebb and flow), and a low input, high throughput system (shadehouse-based ebb and flow). Projects for 2009 are designed to uncover physiological artifacts that appear to have confounded previous grapevine salinity screening projects.

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

Breeding Grape Rootstocks for Lime and Drought Tolerance

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Grapevine rootstock breeding commenced in the 1970s at the Georgikon Faculty of Pannon University by using genetic resources from a collection of 118 rootstock genotypes. The main aim was to develop highly lime tolerant rootstocks, because approximately one-half of Hungary's grape regions suffer from lime-induced chlorosis. The lime content in some of these soils even precludes the use of the Teleki rootstocks. To begin the program the rootstock collection was characterized by morphological traits and analysis of DNA. Next, parents were chosen to accentuate lime tolerance and ease of propagation. As the program progressed drought tolerance was also added to the suite of breeding goals. Seedling populations were made in the mid-1990s after parents had been characterized and selected. Their evaluation continues. After initial morphological evaluation, 96 genotypes (93 rootstocks and 3 *Vitis vinifera* L. cultivars) were evaluated with 19 SSR primers. The primers were chosen from each chromosome to give well-defined heterozygosis and to begin the process of developing a genetic linkage map on which lime and drought tolerance could be mapped. The results of this preliminary SSR analysis show high heterozygosity and polymorphism among the genotypes and a broad range of diverse SSR alleles among the rootstocks and *V. vinifera* cultivars.

Funding support: Hungarian Scientific Research Fund (project no. K 73708).

Rootstock Influence on Cabernet Sauvignon and Merlot Vine Performance in California's Central Coast

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A two-year study was conducted in an irrigated vineyard near Paso Robles, California to evaluate the effects of six extensively used rootstocks—5C Teleki (5C), SO4, 3309 Couderc (3309C), 140 Ruggeri (140R), 110 Richter (110R), and 1103 Paulsen (1103P)—on the viticultural and enological performance of Cabernet Sauvignon (clone 7) and Merlot (clone 3) grapevines (*Vitis vinifera* L.). The experimental design was a randomized complete block design with each block of six rootstocks on one cultivar

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replicated six times. Viticultural performance measurements during the two seasons (2007 and 2008) included vine vegetative growth (shoot number per vine prior to shoot thinning and pruning weights), vine yield components (cluster number per vine prior to shoot thinning, cluster number at harvest, yield per vine, cluster weight, number of berries per cluster, and berry weight), seasonal vine nutrient status (total N, NO3-N, P, and K concentration in petioles/blades at bloom, veraison, and harvest), and leaf water potential (midday Ψ_L measurements prior to irrigation events). Enological performance measurements during the same two seasons included grape must composition for grapes harvested at the same soluble solids levels (pH, titratable acidity, yeast assimilable nitrogen, ammonia, and potassium) and wine phenolic composition (tannins, total anthocyanins, polymeric anthocyanins, and catechin). Preliminary results indicated significant differences among the rootstocks on vegetative growth measurements (pruning weights/Ravaz index) and yield components (yield per vine and cluster weight). Grape must composition also varied significantly among the treatments. although vine seasonal nutrient status was not significantly affected by rootstock selection.

Funding support: ASEV scholarship, California State University, Fresno, J. Lohr Vineyards and Wines, and Kearney Agricultural Center.

Location and Rootstock Effect on Free Amino Acid and Phenolic Composition of Grape Cultivars

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It was previously shown that Merlot fruit from the Oakville Experimental Station in Napa Valley grown on 1103P accumulated 1.7 times as much free amino acids as fruit from 101-14 Mgt with no significant effect on tannin, iron-reactive phenolics, or anthocyanin. Berries sampled at commercial maturity were frozen, the skin was removed, and the seeds separated from mesocarp tissue. Components were ground in liquid nitrogen and extracted with 80% EtOH for amino acid analysis or 70% acetone for phenolic analysis. Free amino acids were determined by HPLC. Protein precipitated tannin and iron-reactive phenolics were measured using ferric chloride, and anthocyanins were assayed by pH change. This study confirmed the observation with Merlot at the Oakville site and showed the rootstock effect on amino acid accumulation in the fruit to be reproducible between years. Samples of Pinot noir (San Louis Obispo) and Sauvignon blanc (Monterey County) showed a similar effect, but Cabernet Sauvignon (Oakville Station), Syrah (Washington State), and Chardonnay (Washington State) showed the opposite effect, that is, greater accumulation of amino acids in fruit from 101-14 Mgt grafted vines. Chardonnay grown in Monterey County showed no appreciable rootstock effect on free amino acid content. The effect of rootstock on amino acid accumulation in the fruit appears to be variable and perhaps dependent on rootstock-soil interactions. We also observed a small but consistent increase in phenolic composition (expressed as per gram berry fresh weight) in fruit grown on 101-14 Mgt across locations, not observed in the previous study.

Funding support: American Vineyard Foundation and the Viticulture Consortium.

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Use of Carbon Isotope Discrimination on Grape Sugar as a Tool for Practical Vineyard Management

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Vine water status has a major impact on grape composition and wine sensory attributes. Many techniques have been developed over the years to assess vine water status. Most of them need extensive field measurements, which limit their practical use for vineyard management. Carbon isotope discrimination ($^{13}C/^{12}C$ ratio, or $\delta^{13}C$) measured on grape sugar at harvest time is an integrative indicator of vine water status. It can be measured by mass spectrometry on grape juice and it does not need any intervention in the field other than grape sampling at harvest. Hence, many measurements can be carried out at a reasonable cost, making this method available for vineyard management purposes. In irrigated vineyards, high-quality fruit is produced with fine-tuned deficit irrigation. $\delta^{13}C$ measurements on grape sugar allow validating irrigation strategies by showing which plots received too little or too much irrigation water. $\delta^{13}C$ measurement provides wineries that purchase grapes with a practical tool to assess if deficit irrigation was implemented in the vineyard. Grape quality for red wine production is closely related to the level of water deficit the vines have been subject to during the growing cycle. $\delta^{13}C$ could be used as an indexation tool for establishing the price of the grapes. In dry-farmed vineyards, $\delta^{13}C$ values are closely related to the water-holding capacity of the soil, canopy architecture, and the climate of the vintage. Vine water status is a major component of terroir expression. Because many measurements can be carried out at a reasonable cost, $\delta^{13}C$ is a powerful tool to map vine water uptake conditions and can thus be used in terroir zoning studies. Vine water deficit promotes shoot-growth slackening, reduces berry size, and increases grape anthocyanin content. Hence, these parameters are well-correlated with grape $\delta^{13}C$ measured at ripeness.

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Evaluation of Potassium during Commercial-scale Red Wine Fermentations and Model Wine Extractions

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Potassium is the most abundant inorganic element found in wine and is present in the skins, seeds, and pulp of the grape. Potassium forms soluble and insoluble salts with the major organic acids found in grapes and plays a significant role in determining wine pH. It is generally thought that potassium is extracted from the various tissues during fermentation, although little data exists to support this assumption. Commercial-scale fermentations were carried out that varied contact time and finished alcohol content. Comparison of treatments showed the control had statistically greater potassium concentration than the high ethanol and extended maceration treatments. Potassium was measured throughout the fermentations and peaked after crushing, diminished during the period of skin contact, and thereafter remained constant (up to 161 days). Comparison of grape and pomace potassium contents revealed that a small amount of potassium is extracted from the seed during fermentation, whereas skins showed adsorption of potassium during the same period. Model wine extractions from grape skins showed that potassium extraction increased by lowering pH, suggesting that negatively charged polysaccharides in the grape skins may be forming ionic bonds with potassium. Model wine extractions of grape skin showed that the combined effect of ethanol and potassium concentration both limited the extraction of skin potassium and increased potassium adsorbed to the skin than in original fruit. The model solution work demonstrated that the key factors in retaining and adsorbing potassium in skins were ethanol and potassium concentration in must/wine or model solution.

Funding support: Washington Wine Grape Funds.

Naturally Occurring Spatial Variability in Canopy Biomass Impacts Flavor and Aroma Compounds in Riesling

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Naturally occurring spatial variation within vines can create localized variability in canopy biomass distribution and microclimate even among neighboring vines that are subjected to the same trellising, training, and cultural practices. To investigate the influence of this "nanoclimatic" variability on fruit chemistry, we selected 12 panels from each of three blocks of commercially managed Riesling in the New York Finger Lakes. Canopy architecture and biomass distribution were measured using the recently developed methods of enhanced point quadrat analysis and calibrated exposure mapping for all panels at veraison. The fruit from each panel was individually harvested and pressed. Panel juice samples were blended to create treatments of low, medium, and high average cluster exposure (22%, 34%, and 40% ambient sunlight, respectively). The resulting juice was quantitatively analyzed by GC-MS to measure levels of free and bound aroma compounds, including *cis*-3-hexen-1-ol, linalool, α-terpineol, β-damascenone, geraniol,

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and phenylethyl alcohol. Results revealed substantial variability in panel-level average cluster exposure within the same block (mean 32% of ambient light, ±8%, range 15–46%). Analysis revealed correlations between increasing average cluster exposure in the blended juice treatments and concentrations of both *cis*-3-hexen-1-ol (-55%, $p < 0.05$, r = 0.66) and α-terpineol (-12%, $p < 0.01$, r = 0.65). The quantitative nature of the results has implications for improved precision and economic efficiency of cultural practices aimed at influencing fruit quality.

Effect of Yeast Composition and Vinification Strategy on Sensory Perception and Flavor Composition

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The aim of this study was to clarify the correlation between terroir and the corresponding yeast composition naturally occurring in the vineyard. Almost the same factors influence the character of wine and the survival and composition of yeast, for example microclimate, soil, and green-cover. In cooperation with different wineries, six terroir sites were selected and hand-harvested separately under sterile conditions. Some of the grapes were spontaneously fermented in the wineries, including natural yeast flora from the vineyard and established "cellar yeast" found on tanks and working material. Other grapes were processed under sterile conditions to exclude other microorganisms than those from the vineyards. The different yeast genera were identified by PCR and DGGE to find differences depending on their origin. Analytical "fingerprints" of the wines were produced by GCxGC and MS detection to find differences in flavor composition that could be related to yeast composition and activity. Results show that fermentation strategy and cellar strains are of greater importance to the outcome of fermentation than the differences in yeast composition in the vineyards. It also became obvious that there is a connection between fermentation time and flavor perception in spontaneously fermented wines. On a sensory and analytical basis, wines produced by traditional fermentation strategies like *pied de cuve* could not be distinguished from those fermented by dried active yeast. The results lead to optimized procedures for spontaneous fermentation that combine rich flavor composition from different yeast genera with a higher fermentation safety.

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Role of Yeast Sulfur Metabolism in the Formation of Hydrogen Sulfide from Elemental Sulfur

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The presence of elemental sulfur in grape must is generally believed to result in the production of H_2S during the primary fermentation due to the reductive conditions created by yeast metabolism. Quantification of the amounts of H_2S formed from elemental sulfur is difficult because yeast sulfur metabolism also contributes to the appearance of H_2S. The goal of this study was to evaluate the impact of elemental sulfur addition on strains with differing abilities to reduce sulfate to sulfide. Strains deficient in sulfite reductase were generated and tested for the appearance of H_2S in synthetic and natural juices directly spiked with elemental sulfur. These media contained sufficient natural levels of methionine to allow equivalent growth and fermentation of the strains. Derivatives of UCD522 carrying single gene modifications at the *MET10* locus were used: 117 (*met10Δ/met10Δ),* 146 (*met10Δ/MET10-932*), 144 (*met10Δ/MET10-522*), 145 (*met10Δ/MET10-288*), and UCD522 (*MET10-522/MET10-522*). Strain 117 does not contain a functional sulfite reductase and strain 146 contains an allele of sulfite reductase that does not lead to sulfide release. These strains behaved very similarly: trace amounts of H_2S were formed at the lowest level of inorganic sulfur with higher detectable levels produced as the level of elemental sulfur increased. The other three strains produced much higher H_2S levels under all conditions and there appeared to be only a modest impact of the presence of inorganic sulfur. This study confirms the production of hydrogen sulfide in the absence of yeast sulfur metabolism, but at low levels.

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

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Viticulture and Climate

Updated Analysis of Climate-Viticulture Structure and Suitability in the Western United States

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While tremendous advances have occurred in spatial climate data products, no large-scale update to our understanding of climate-viticulture structure and suitability for the western United States has been done in the last three decades. The goal of this research is to better document the climate structure in wine-producing regions throughout California, Oregon, Washington, and Idaho, making comparisons between regions in the U.S. and elsewhere in the world more realistic. The research uses the 1971-2000 PRISM 400m climate grids to assess four climate parameters commonly used to characterize climate suitability for viticulture and wine production: the Winkler index (degree-days), the Huglin index, the biologically effective degree-day index, and average growing season temperatures. The data are assessed and depicted as spatial averages of the climate parameters over 135 American Viticultural Areas in the western U.S. The method takes into account the entire region (not individual stations) and the relative relief of the growing regions to provide a much better representation of the spatial climate suitability in each region. The research also provides the first comprehensive comparison of the Huglin index in the U.S. and develops greater latitudinal adjustment factor for calculating the index that can be applied elsewhere. The methods developed in this work are also being applied to other wine regions throughout the world (Europe, Australia, New Zealand, South Africa, and South America) with the goal of producing a worldwide set of similar data in which to make appropriate climate comparisons.

Weather, Climate, Characteristics, and Prices of California Winegrapes

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Prices of winegrapes grown in California differ dramatically across climatic regions and varieties. Surprisingly there are only few studies that empirically estimate relationships among climate, weather, fruit composition, and price. A biophysical relationship between weather and harvest Brix is known to exist, although the relationship varies across varieties, soil, and topography and may be controlled to some extent by winegrowing practices. In some cases prices are determined before weather and specific characteristics of grapes are known. Climate affects the price of winegrapes mainly through long-term reputation, and weather affects vintage-to-vintage spot prices. We use time-series cross sections of weather and winegrape data over several decades to model and document

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the effect of weather and climate on characteristics and prices of winegrapes grown in 17 crush districts in California. Estimation is divided to three parts: weather and Brix relationship, Brix and price relationship, and weather, climate, and price relationship. Each crush district is considered a separate market. Nonetheless, grapes grown in different crush districts in California are in direct competition and their price is jointly determined. Therefore weather, climate, and grower adaptation to weather and climate in each region affect the price of winegrapes grown in competing regions. We use these historical relationships to simulate the potential effects of climate change on California winegrape industry.

Vineyard-scale Climate Variability, Vine Light Intensity, and Pinot noir Phenolic Composition

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We conducted a three-year field study to correlate tannin, anthocyanin, and iron-reactive phenolic concentrations from commercial Pinot noir vineyards in the Carneros and Sonoma Valley appellations with climate statistics derived from hourly temperature measures from each vineyard site. We used several statistical approaches to identify key phenologically based periods influencing phenolic concentration at maturity, including correlation and regression trees, factor screening, principal components analysis, and pairwise correlations. The results from these statistical models suggested that cool conditions in the fall the year before harvest, warm temperatures from budburst to bloom, and cool temperatures from bloom to veraison were positively correlated with concentrations of all three classes of phenolics, although not all trends were significant. Anthocyanins were positively correlated with moderate temperatures from veraison to harvest. Tannins were also significantly increased by warm nights preceding budburst and warm days from budburst to bloom. We measured relatively high levels of light interception (for n = 561 vines, the mean interception of photosynthetically active radiation was 36%) and found that increased light levels were significantly correlated with lower levels of all three classes of phenolic compounds in this study.

Funding support: David and Lucile Packard Foundation Stanford Graduate Fellowship and the School of Earth Sciences, Stanford University.

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Gas Exchange of Field-Grown Cool, Warm, and Hot Climate Grapevine Varieties in West Texas

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Throughout growing seasons in west Texas, grapevines are subjected to a variety of temperature extremes. Therefore, to assist growers when selecting adapted varieties, evaluations must be conducted under climatic conditions found in the region. This research evaluated gas exchange of field-grown (planted spring 2006) *Vitis vinifera* varieties subjected to variable leaf temperatures. Cool climate (Pinot noir), warm climate (Cabernet Sauvignon and Merlot), and hot climate (Grenache and Mourvedre) varieties grafted to 110R rootstocks were arranged in a randomized complete block design with four blocks and five vines of each variety within each block. On seven dates during the 2008 growing season, midday photosynthetic rate, stomatal conductance, and leaf-to-air vapor pressure deficit were measured with a LI-COR 6400 on two leaves from three vines within each block. Measurements were made at leaf temperatures ranging from 24 to 40°C. Leaf chamber light levels were maintained near 2,000 PAR. Gas exchange measurements at low leaf temperatures indicate Grenache and Mourvedre (hot climate varieties) had lower gas exchange rates when compared to other varieties. However, our data suggest at greater leaf temperatures Cabernet Sauvignon (warm climate variety) generally maintained gas exchange at the greatest rate. In addition, gas exchange levels for Mourvedre at high leaf temperatures were less when compared with other varieties. Additional research through the 2009 growing season will give further insight to the adaptability of these varieties to the west Texas climate.

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Effects of Pulsed Energy Field Treatments on White Winegrapes

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The effects of pulsed electric field (PEF) treatments on Chardonnay (Carneros AVA), Sauvignon blanc (Carneros AVA), and Trebbiano Toscano (Oakville AVA) were studied in 2008. Electroporation of the cell membrane via PEF has been demonstrated in grapes. In this study an electrical field was generated which was applied to grapes after gentle crushing. Three main treatment types were performed using three different pulse durations of 12 ns, 200 ns, and 2000 ns. Further, the number of pulses administered for each pulse length was varied by using 100, 200, and 800 pulses. Application of PEF was shown to increase juice yield 30% in Chardonnay, 35% in Sauvignon blanc, and 20% in Trebbiano Toscano. Interestingly, the PEF treatment that attained maximum juice yield was different for each variety. Spectral measurements on juice at 280 nm (total phenols) and 420 nm (browning) were recorded and individual phenolic compounds were analyzed by HPLC. Most treatments generally reduced browning with statistical significance compared to the control. This was confirmed by visual observation. While browning was reduced, phenolic content measured by UV absorption at 280 nm and several individual phenolic compounds quantified by HPLC were significantly increased. In this study the application of PEF demonstrated significant impacts on juice yield and quality. Presently, PEF technology is used for various functions in the food industry. Benefits to the wine industry, including economic, are foreseen.

Inhibition of Powdery Mildew by Improved Vineyard Sunlight Exposure

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It is commonly observed that severity of grapevine powdery mildew, caused by *Erysiphe (Uncinula) necator*, is lower on tissues exposed to full sunlight than those in the shade. However, the magnitude of this effect and its causes has received little study. We have decreased the severity of powdery mildew on fruit by up to 50% and on leaves by up to 87% compared to those in shaded conditions by manipulating the vineyard canopy to promote sunlight exposure. Environmental monitoring of the mesoclimate showed no differences in ambient air temperature or relative humidity in the sunny versus shaded portions of the canopy. However, the midday surface temperatures of fully exposed leaves were 5 to 15°C higher than those of shaded leaves and frequently in a range detrimental to fungal development or survival. Furthermore, ultraviolet radiation (UV-B) levels in the shaded areas averaged 90% less than in the direct sun. In subsequent experiments, a UV-filtering material was placed over sun-exposed vines to provide tissues that were heated by the sun but protected from UV radiation. On these vines, disease severities on leaves and clusters were intermediate between the shaded (most severe) and sun-exposed (least severe) vines, suggesting that sunlight limits disease development through

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at least two different mechanisms harmful to *E. necator*—high substrate temperature and exposure to UV radiation. Increasing sunlight exposure of fruit via leaf removal at fruit set and using a vertical shoot-positioned training system reduced disease severity by 35% relative to Umbrella-Kniffen trained vines without leaf removal.

Effect of ABA on Vine Physiology and Grape Quality of Cabernet Sauvignon in a Cool-Climate Area

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The Niagara region, where most of the Canadian wine industry is located, has to deal with high climate variability. In cool years, veraison occurs later and grape ripening problems occur. Recently, abscisic acid (ABA) has been associated with the main biochemical processes during grape berry ripening. One of our hypotheses was that by applying externally ABA, we can hasten veraison and improve grape quality. Two experiments were set up in one Cabernet Sauvignon block, located on Niagara on the Lake, Ontario, Canada. The first experiment had four treatments consisting of full canopy, clusters, and leaves sprayed plus an untreated control. The second experiment had three treatments represented by rates of 150 and 300 ppm and untreated control. Three replicates were assigned per treatment for each experiment. In each row replicate, the treatments were randomly assigned and equally distributed. Berries and leaf samples were collected after each treatment. Transpiration and water leaf potential were also recorded. At harvesting, yield components and berry composition were analyzed. After four weeks from experiment initiation, clusters from control still had 20% green berries. The ABA application hastened the onset of veraison, having almost the same effect in all treatments. However, the magnitude of physiological effects varied with ABA concentration and its uptake rate in the vine. The highest uptake rate of ABA was found on leaves and the lowest on berries. Lower rate of ABA applied on whole canopy had a better effect on fruit quality then high rate applied only on clusters.

Greenhouse Testing Conditions Can Alter Expression of Pierce's Disease Resistance

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The long-term solution to Pierce's disease (PD) is resistant cultivars. A PD-resistance breeding program has been underway for many years at UC Davis. This program depends on a very specific greenhouse-based evaluation for resistance, which can be greatly influenced by environmental conditions. A series of three experiments was carried out to examine alterations in the expression of PD resistance under greenhouse conditions, which included the effects of pot size (6-cm sub-irrigated and 10.5-cm drip-irrigated), bacterial inoculation level (10^4, 10^5, 10^6, and 2.5 x 10^6 cfu/mL cell suspension), and length of postinoculation period (7, 10, and 13 weeks) on symptom development, and pathogen populations. The impact of cutting back the shoots prior to inoculation was

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also tested. A second experiment used three pot sizes (including an intermediate size). The vertical height and spacing of the vines were also altered to assess the role of leaf area and plant density resistance expression. A third experiment tested the effects of shading and light availability on pathogen populations. Results showed a significant effect of cutting back before inoculation, length of postinoculation period, pot size, plant density, and light level on pathogen populations in stem tissue of resistant genotypes. Inoculation level had no effect above a threshold inoculum level of 10^5 cfu/mL (2,000 cells).

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Phytotoxicity of 2,4-D Spray Drift to Grapevine: Symptomatology, Micromorphology, and Cytology

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The auxin hormone mimic 2,4-D (2,4-dichlorophenoxy acetic acid) is generally not used in grape production due to its herbicidal properties specific to plants with broad leaves; however, grapevines are injured by 2,4-D, which enter the vines through spray drift and vapors. The objective of this study was to elucidate the symptomatological, cytological, and micromorphological characteristics of 2,4-D injury to grapevine. Shoots with phytotoxicity symptoms of 2,4-D displaying zigzag internode growth were identified from a vineyard. Tissues from injured leaves were prepared for examination with light and electron microscopy. The leaves affected by 2,4-D were grotesque, ranging from fan to funnel shape with thick fasciated bleached veins and pointed leaf margins. Leaves were puckered with raised areoles on the adaxial surface and correspondingly deep grooves on the abaxial surfaces. The trichomes, petioles, and the compound buds in its axil appeared to be healthy. The adaxial epidermal cells were enlarged; some of them underwent periclinal and tangential divisions. The abaxial surface developed unevenly because of excessive proliferation of cells. Within an areole there existed a malformed palisade parenchyma as tightly packed oval-shaped cells with no air spaces. The stomatal morphology and its position were altered; they were not coplanar to the epidermal surface and an irregular growth extended from the upper rim of the stomatal pore. Grapegrowers and winemakers can use the knowledge of morphological and cellular modifications as a diagnostic tool to identify 2,4-D injury and rectify it using appropriate measures.

Chelating Agents: A New Tool in Preventing Wine Oxidation

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Oxidation affects the stability and sensory proprieties of wines, and thus, it is a major concern in the winemaking process. The chemical bases of wine oxidation are still not completely understood. The knowledge of the mechanisms underlying wine oxidation

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will potentially help with reducing wine deterioration and achieving better results when a moderate wine oxidation is desired in order to stabilize color and reduce astringency. Our research focuses on the role of oxygen and metal catalysts in wine oxidation. Trace metals are present in wine and have been proven to initiate chemical oxidation of wine components. We hypothesize that the addition of chelating agents to wine will inhibit oxidation by sequestering metal catalysts. Results in our laboratory confirmed that the presence of iron (5 mg/L) and copper (0.15 mg/L) accelerated acetaldehyde formation and oxygen depletion, markers of oxidative processes in model wine solutions. We report the effect of a number of different metal chelators (e.g., ethylenediaminetetraacetic acid, ferrozine, and phytic acid) on wine oxidation. A decrease in oxygen concentration is used as a marker for oxidation. Both model wine solution and red wine containing chelator and catalytic trace metal concentrations were monitored for oxygen depletion using fluorescent sensor dots. The goal of this research is to provide winemakers with effective tools for controlling wine oxidation.

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Impact of Heat, Ethanol, and Acid Preadaptation on Viability of *Saccharomyces cerevisiae* after Drying

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This study was aimed at improving viability of winemaking yeast *Saccharomyces cerevisiae* after spray and freeze-drying processes by preadapting yeast to moderate heat, ethanol, and acid stresses. *Saccharomyces cerevisiae* UP3OY5 strain was harvested in the middle of stationary phase and spray dried at an inlet temperature of 130°C and an outlet temperature of 60°C with carriers such as skimmed milk, maltodextrine, and sucrose. Use of 10% (v/v) skimmed milk as a carrier gave the highest viability (8%) of the three treatments of nonadapted cells. Before yeast cells were spray dried, long-term adaptation was performed by cultivating yeast in the presence of 8% ethanol (v/v) or at acid pH (3.5) and short-term adaptation was performed by shocking yeast at 45°C for 60 min. Adaptation to ethanol appeared to be most effective in improving yeast viability. The viability of ethanol-adapted yeast was five-fold higher than that of nonadapted yeast after spray drying with maltodextrine as a carrier but remained low (only 16% of viability recovered after spray drying versus 3.0% without adaptation). Therefore, spray drying did not seem effective as a dehydrating method. The same adaptations were used before freeze-drying yeast in order to increase yeast viability. The influence of heat, ethanol, and acid preadaptation on changes in membrane composition was also investigated. Fatty acid profiles of the membranes of adapted yeasts were analyzed by gas chromatography and the membrane physical state was assessed by differential scanning calorimetry.

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Brettanomyces bruxellensis Incidence and Diversity in Italian Wines as Determined by Molecular Methods

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The yeast *Brettanomyces* produces volatile phenols and acetic acid. The uncontrolled accumulation of these molecules in wine leads to sensorial defects that compromise wine quality. In this work we present a multidisciplinary approach for the investigation of *Brettanomyces bruxellensis* presence in wine and the correlation between ethyl phenols content and *Brettanomyces* ecology. For the detection and quantification of *B. bruxellensis* in 87 different wines from Liguria and Piedmont regions in northwest Italy, we have applied qPCR directly in the wine, as a culture independent method, as well as a traditional culture dependent method to define the incidence of *B. bruxellensis*. Furthermore, headspace solid-phase microextraction was used to quantify ethyl and vinyl phenol in wines. Finally, molecular characterization of the 217 *B. bruxellensis* isolated strains was performed for a better knowledge of strain biodiversity. The combination of these methods led us to the conclusion that an increase in the *B. bruxellensis* population is not directly correlated to an increase in vinyl and ethyl phenol content.

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Effect of Interaction between Yeast and Malolactic Bacteria during Simultaneous Inoculation in Must

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Malolactic fermentation (MLF) can be indispensable for the production of red and white cool-climate wine. It leads to a deacidification and to aroma modification, and consequently improves the sensory quality of wines. For these reasons it is encouraged either during the final phase of alcoholic fermentation or after its conclusion. Malolactic bacteria (MLB) can be added after completion of alcoholic fermentation (AF) of yeasts or in simultaneous inoculation. The use of simultaneous inoculation could lead to stuck AF, to the increasing of acetic acid concentration because of the yeast/bacteria competition in the must; in contrast, the inoculation of MLB after the AF could remain difficult to achieve because of the inhibitory effect of ethanol and acidity of wines. Considering that the simultaneous inoculation of yeast and MLB in must remains controversial, the aim of our work was to evaluate its effects on fermentation kinetics and key wine parameters (ethanol, acetaldehyde, glycerol, lactic and acetic acids) of Moscato must. Four commercial yeast and four MLB were tested in 16 combinations in Moscato must. Results demonstrated that the simultaneous AF/MLF did not have any negative impact on fermentation kinetics and success or on final key wine parameters. Acetic acid concentration was low in all the combinations and the fermented products obtained were acceptable for consumption. Significant differences were found with regard to the yeast/bacteria growth, final pH, total acidity, and ethanol, lactic acid, and glycerol concentrations among the yeast/bacteria combinations.

Effect of Aging on the Sensory Properties of Pinot noir Wines

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Twenty commercial Pinot noir wines from the 2001, 2002, 2003, and 2004 vintages were evaluated by descriptive analysis by trained panels, first when they were released on the market (in 2005) and then four years later (in 2009). The basic composition of the wines (e.g., residual sugar, pH, titratable acidity, ethanol, tannins, color) was also measured by instrumental means. The matrices of mean attribute intensity ratings across the wines were analyzed by cluster analysis (CA), principal component ananlysis (PCA), and canonical variate analysis (CVA), and the relation between the instrumental and sensory measures was investigated with partial least square (PLS) regression. We also used regression techniques to find predictors of aging patterns among the original instrumental and sensory measures. The number of sensory attributes evaluated increased from 17 in the original descriptive analysis to 25 for the aged wine evaluation, indicative of the new sensory qualities that emerged upon aging. Overall, fruity characteristics, astringency, and red color subsided with aging and were replaced with attributes indicative of phenol polymerization or, for a number of wines, oxidation. The

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off-flavors found in some of the original wines increased upon aging. For the most part, the wine cluster structure derived from the cluster analysis was retained upon aging. Those wines with sound sensory, chemical, and microbiological bases of fruity attributes, high phenols, and no bacterial or yeast contamination at release proved to have the most desirable sensory profiles upon aging.

White Grape Extended Skin Contact Winemaking Technique

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Traditional white wine vinification involves pressing before fermentation. In some cases, prefermentative maceration is performed and it rarely continues during fermentation. In this work, an extended maceration was conducted in order to produce diversified white wines. In recent years different tests with three vinification protocols using Garganega grapes in Verona (Italy) were performed. The maceration period was protracted up to 4 months without adding adjuvant products. Wine was chemically analyzed during vinification and aging. Sensory analysis was performed, comparing extended skin contact with a standard white test wine produced from the same grapes. Wine, during maceration, must be kept in a reductive environment to avoid polyphenol monomer oxidation. In addition, the cap, which is easily oxidizable, must be gently treated (few and low volume pumpovers) and the environment must be periodically flushed with an inert gas, nitrogen or argon, after fermentation. The use of the extended skin contact technique yields wines with different chemical and sensory profiles and, even if the high polyphenol content makes the wine sensitive to oxidation, results in interesting complex flavor and structure.

First Crushing Experiences in an Inert Environment

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Among prefermentative processes used with white wines, the extraction and the separation of juice are critical phases. During the time interval between the berry breaking (crushing or pressing bunch grapes) and the start of alcoholic fermentation, the juice undergoes deep modification because of the action of endogenous and exogenous enzymes. Enzymatic activity oxidizes not only the phenolic fraction but also the aromatic compounds, thereby limiting the varietal expression of wine. Conventional intervention uses sulfur dioxide, inert gas, and the drop of temperature of either the grapes and/or of crushing and/or juice temperature. The use of sulfur dioxide and/or ascorbic acid partially resolves the problem with oxidation. Use of nitrogen or other inert gas for flushing presses reduces must oxygen content. In this research a pilot crush and destemmer (Kappa Neutral) was used; it was flushed with nitrogen and fed with a protected conveyor belt. Flushing with an inert gas takes the crushing chamber oxygen value to 3.5%. Three vinification protocols, using sulfur dioxide and ascorbic acid, as sole addition and mixed together, were tested. A reduction in dissolved oxygen content in the inert gas flushed control compared with the nonflushed conventional crushing

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was observed. Analytical data confirmed the higher oxidation in the traditional process; while in the inert system, a higher protection of polyphenolic compounds was observed. Less oxidation was also observed for the most oxidizable components, like hydroxycinnamoyl tartaric acid and catechins.

Botrytis cinerea Noble Form Induction on Grapes during Withering

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Botrytized wines are very important for their complex and distinct aroma. These wines usually come from overripened grapes. Grapes can be overripened on the plant or in proper drying rooms. *Botrytis cinerea* grows in noble form only in specific conditions which are not present in every environment. Environmental temperature and humidity are changing. Also in the Venetian area (Italy) drying rooms, the incidence of noble *Botrytis* is less than it was 20 years ago because of climatic changes and less foggy humid days during the withering process. In this research *Botrytis cinerea* noble form was induced by setting proper wetting, humidity, and temperature conditions in drying rooms. In this way, it is possible to produce botrytized wines from dried grapes in traditional areas and also in new zones where climatic conditions do not allow the natural *Botrytis* infection.

Effect of Viscosity on the Perception of Sweet, Sour, Bitter, Ethanol, and Viscous Mouthfeel

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A system of model wines was used to study the interaction of instrumental viscosity on the perception of ethanol, acidity, and viscous mouthfeel. Two levels of tartaric acid (3.5 g/L), two levels of ethanol (10%, 13% v/v), and three levels of measured viscosity (1.6, 1.8, 2.0 cP at 20°C) were selected to create 12 model wines. Using standard sensory methods, a panel of 12 judges rated each model wine, in triplicate, for sweet, sour, bitter, perceived ethanol, and viscous mouthfeel. The results from the sensory analysis were analyzed using principal component analysis (PCA) and means separation. The PCA explained a total of 91% of the variation within the first two components. The first component accounted for 52% of the variation and separated the model wines in the positive direction by ethanol and bitter and in the negative direction by viscous mouthfeel and sweet. The second component accounted for 39% of the variation and was driven by sour in the negative direction. Overall, increasing the instrumental viscosity decreased the perception of sour, bitter, and ethanol, while increasing the perception of sweetness and viscous mouthfeel.

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Prevention of Grapevine Disease Using Antagonistic Microbes

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Bacteria with antagonistic activity against *Botrytis cinerea*, the pathogen responsible for gray mold, were isolated from grape berry skins. We evaluated the effectiveness of these isolates as biological control agents against several types of grapevine disease. KS1 was identified as a new strain of *Bacillus subtilis* according to analysis of its morphological, physiological, and genetic features. KS1 suppressed the growth of *B. cinerea* and also *Colletotrichum gloeosporioides*, a pathogen responsible for grapevine ripe rot. The KS1 isolate was also tested on leaves of *Vitis vinifera* L. cv. Koshu in an experimental vineyard. Application of the isolate resulted in a decrease in the frequency and area of grapevine downy mildew caused by *Plasmopara viticola.* In addition to the isolate adapting well to grapevines, our results suggest that KS1 has an advantage over commercial biological pesticides in that it prevents a broad range of grapevine diseases in vineyards, whereas commercial products are effective against only *B. cinerea*. Therefore, we propose that the use of these antagonistic microbes would contribute to the further improvement of integrated pest management systems against grapevine diseases and potentially to reducing the amount of agricultural chemicals required for vineyard treatment.

Optimization of Wine Flavor by Masking and Enhancing Target Flavor Compounds

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The presence as well as the absence (in case of off-flavor) of particular aroma compounds in a well-balanced manner modulates the final overall perception and appreciation of alcoholic drinks. Typically, in alcoholic beverages these aroma compounds are formed during complex fermentation processes and can be analyzed using specific chemical techniques. Both the static release (orthonasal perception) and the dynamic release (retronasal perception) of aroma compounds are essential. In vivo techniques such as olfactometry, gustometry, olfactoscan, MS-nose and GC-O can be used to study these phenomena in an in vivo manner. We hypothesize that, if you are able to understand the aroma-aroma, aroma-taste, and aroma-texture interaction during consumption of wine, it is possible to optimize its flavor by making use of selected starters. An in vivo analytical technique (olfactoscan) is used to study aroma-aroma interactions in a white wine to mask an off-flavor. A microscale alcoholic fermentation setting in a real product matrix is shown for the selection of starters, which are able to produce the necessary masking compound. This approach will allow a very selective optimization of wines.

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Influence of Berry Dehydration on Berry Fresh Weight and Fruit Composition during Hang Time in Cabernet Sauvignon Grapes in a Warmer Region

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Hang time has been practiced by some in recent years as a means to obtain more fully developed flavors to enhance wine quality. Higher Brix, lower yield, and berry shriveling are usually observed with later harvest and hang time in warm regions. Berries of Cabernet Sauvignon, grown using conventional drip irrigation and preveraison regulated deficit irrigation in Fresno, CA, were sampled with no, slight, medium, or severe shriveling in 2007 and 2008 to investigate the impact of berry dehydration on berry weight and fruit composition. Berries without visual shriveling did not change much in their weight and composition during hang time. Irrigation, sampling dates, and cluster location in the canopy had only minor influence. However, berry dehydration associated with visual shriveling had profound impact on berry weight and fruit compositions. Increasing degree of berry dehydration resulted in higher Brix, TA, and pH, but reduced fruit fresh weight and total anthocyanin content approximately two-fold. Wine extractable anthocyanins, phenolics, and tannins were reduced 28-, 3-, and 8-fold, respectively, when berry water content decreased from 68% to 52 or 32%. The number of dead pedicels increased from 1 to 2% for berries without any shriveling up to 100% when berries were dried to raisins. Hang time resulting in Brix higher than 26 seemed to reduce yield and fruit quality while concentrating sugars and acids. This most likely will affect wine quality negatively and should be avoided in warm regions.

Funding support: California State University, Agricultural Research Initiative Grants Program.

Effect of ABA Application to Enhance Fruit Color on Vine Vigor, Yield Components, and Fruit Composition in Cabernet Sauvignon Grapes in a Warm Region

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It is well established that ABA application improves fruit color in grapevines. Recent research reports have demonstrated up to 100% increase in fruit anthocyanin concentration when clusters were sprayed with ABA to runoff in warm regions. Experiments were conducted in 2006–2008 to determine the effect of ABA on vine vigor, yield components, and fruit composition when applied to enhance fruit and wine color in Cabernet Sauvignon in the San Joaquin Valley of California. No phytotoxicity symptoms were observed from ABA application up to 1200 mg/L. Shoot number, fruit set, cluster number, cluster

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weight, yield, and pruning weight were not affected by ABA applications. Berry fresh weight was increased by some ABA treatments but only at a small magnitude. Earlier ABA application at the beginning of berry softening or coloring resulted in a sharp Brix increase during 4 weeks after treatment as well as lower TA and higher pH at harvest in 2006. However, fruit Brix, TA, and pH were not affected when ABA was applied at fruit coloring or later in 2007 and 2008. Previous reports suggested greater color enhancement in fruit and wine when ABA was applied at 30% berry coloring or later. The results indicate that ABA applications should have no or only very limited influence on vine vigor, yield components, and fruit composition when it is applied to improve fruit and wine color.

Funding support: California State University, Agricultural Research Initiative Grants Program, Constellation Wines US, and Valent BioSciences Corporation.

Phenological Resistance of Grapes to Green June Beetle Damage

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The green June beetle (GJB), *Cotinis nitida* L., is a native insect pest that aggregates and feeds on ripe grapes. The flight period of the GJB peaks in midsummer, when early-season grape cultivars are harvested and midseason cultivars are in the later stages of fruit ripening. The goal of this research is to evaluate phenological resistance; that is, the planting of grape cultivars that ripen outside of the window of peak pest activity as a sustainable strategy for reducing beetle injury to ripe clusters close to harvest. Six cultivars of grapes were evaluated, early-season ripening Foch and Jupiter, midseason Chancellor and St. Croix, and late-season Norton and Chambourcin. By the first harvest date on 7 Aug, nearly all clusters (95%) on Foch, Jupiter, and St. Croix had been entirely consumed by GJB. Chancellor grapes were harvested on 14 Aug and received only 27% crop loss. The late-season ripening cultivars Chambourcin and Norton had very little (5%) or no (0%) damage, respectively. Reduced susceptibility of grape cultivars to GJB feeding is based on phenological asynchrony. Grapes with berries that ripen outside of peak GJB activity are less susceptible to attack and therefore require minimal or no insecticidal control of this pest. Our data indicate that GJB can completely destroy an entire harvest of early-season ripening cultivars of grapes grown in Kentucky if the crop is not protected with sprays. Growers of highly susceptible cultivars should be aware of the limited chemical control options available for near-harvest use.

Funding support: New Crop Opportunities Center–University of Kentucky (USDA Special Grant) and USDA–Southern Region Sustainable Agriculture Research and Education Grant.

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HPLC Analysis of Methanol in Wines and Spirits from Finger Lakes Grapes

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This research was undertaken to analyze the content of methanol in wines and distilled grape spirits made from a variety of American *Vitis labrusca* and French hybrid winegrapes by high-performance liquid chromatography with a Bio-Rad Aminex HPX 87H column and a refractive index detector. Three American *Vitis labrusca* varieties (Concord, Catawba, and Niagara) and three French hybrid varieties (New York Muscat, Vidal, and Vignoles) grown in the Finger Lakes wine region of New York State were used. Each winegrape variety was destemmed, mashed, and fermented under anaerobic conditions with Red Star active dry wine yeast (*Saccharomyces cerevisiae* Montrachet). The concentrations of methanol in the wines varied from 160 mg/L to <350 mg/L, depending on the variety of winegrape fermented. The distilled grape spirits produced by distillation of the six varietal wines were found to have concentrations of methanol ranging from <30 mg to 110 mg per 100 mL 40% ethanol. Use of a commercial pectic enzyme preparation in the wine production process caused a significant increase in the methanol content of the varietal wines and the distilled grape spirits. Results indicate that the concentrations of methanol found in the distilled grape spirits produced from the six Finger Lakes grape varieties were significantly below the U.S. legal limit of 0.35 volume percent methanol for brandy.

Rootstock and Scion Influences on Grape and Wine Composition

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Although phylloxera is not an immediate threat to vineyards in eastern Washington where vines continue to be own-rooted, it still remains a significant threat. Our objective was to determine the effects of rootstock and scion combinations on fruit and wine composition. A field experiment was conducted with three winegrape varieties (Merlot, Syrah, Chardonnay) that were either grown on their own roots or grafted to five different rootstocks (1103P, 5C, 3309, 101-14 Mgt, 140Ru; n = 10). Small-lot wines were made from the corresponding fruit (n = 5). Own-rooted Chardonnay wines had the highest pH and lowest titratable acidity. Merlot wines showed no differences, except 1103P and 140Ru had significantly greater titratable acidity. Own-rooted Syrah had the highest pH and second highest titratable acidity while Syrah grafted to 3309 had the lowest pH and highest titratable acidity. All rootstock scion combinations were high in proline and low in arginine. Only Syrah had significant amounts of arginine and had relatively lower amounts of proline than the other two varieties. No observed differences in alcohol concentration were found for wines within a variety. All of the red wines were evaluated for tannin, anthocyanin, and total iron reactive phenolic content at pressing. The results show that for Merlot there were no significant differences between any of the phenolic components. For Syrah there were no significant differences for the tannins

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and total phenolics; however, some small detectable differences were found for anthocyanins. The rootstocks 101-14 Mgt, 1103P, and 5C had significantly greater anthocyanins than the other rootstocks and own-rooted vines.

Funding support: Northwest Center for Small Fruits.

Effect of Deficit Irrigation on Tannin and Anthocyanin Concentration of Cabernet Sauvignon Grapes and Wines from Washington State

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Regulated deficit irrigation is considered one of the most important viticultural tools for the production of quality grapes and wines. No evidence has been collected in Washington to date about its effect on the important phenolic classes of tannins and anthocyanins, which are responsible for wine astringency and color, respectively. Deficit timing is an important factor because of the timing of biosynthesis for each of the classes of compounds. Four experimental deficits were applied: early season, veraison, full season, and control. The experiment was carried out in a 27-year-old own-rooted Cabernet Sauvignon block near Mattawa, Washington, in a randomized complete block using north-south row orientation with four repetitions of each treatment. Two-hundred berry samples were taken during the season at regular intervals finishing at commercial harvest. Leaf water potentials were taken throughout ripening. At harvest the full and early season deficits had a 10% lower berry weight than the control and veraison treatments but were not different in TA, pH, or Brix. The full and early season deficit treatments had significantly greater skin tannins and anthocyanins on both a berry and weight basis than the veraison treatment and the control. However, on a weight basis the full and early season deficit treatments had significantly greater seed tannins. Wines made from the various treatments (n = 2) show similar results, although the full-season deficit had significantly greater tannins and anthocyanins than the early-season treatment.

Funding support: Washington Wine Grape Funds.

Determination of 4-Vinylcatechol in Wine by HPLC-DAD Coupled with Fluorescence Detection

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The yeasts of the genus *Brettanomyces/Dekkera* are described as the most serious spoilage yeasts in wine because of their ability to produce high amounts of volatile phenols. These compounds, including 4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol, 4-ethylguaiacol, and 4-ethylcatechol, are the cause of one of the most significant problems in winemaking, namely wine off-flavor, which is likened to the smell of wet animal, horse sweat, medicine, barnyard, and leather. We have developed a method that employs HPLC-diode array detection (DAD) with a fluorescence detector to analyze volatile

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phenols, including 4-vinylcatechol (3,4-dihydroxystyrene), in wine. The proposed method accomplishes chromatographic separation in a short time. The method was validated and the detection limit was 20 g/L for 4-vinylcatechol. Then, we applied the method to the evaluation of 4-vinylcatechol concentration in commercial Japanese wines. 4-Vinylcatechol has a phenolic, pharmaceutical, and smoky odor, and its olfactory threshold in water is 1500 g/L.

Effect of Winemaking Conditions on the Formation of Sulfur Taints during Sur Lie Aging

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Sur lie aging contributes positive characteristics to wine, however, sulfur containing off-odors (sulfur taints) may also be produced at the same time, negatively impacting wine perception. The role of juice pH and grape solids content during the primary fermentation on the formation of both H_2S and sulfur taints during sur lie aging was evaluated using a clarified Chardonnay juice. Five yeast strains with different abilities for H_2S production were used to additionally assess the impact of strain background. Wines were aged with and without yeast lees in 750-mL bottles and S-volatile compounds were analyzed by GC. Juice pH had no significant effect on the appearance of S-compounds. The presence of grape solids during the primary fermentation resulted in elevated H_2S production in all five strains. The wines produced from juices with higher solids content aged in the presence of yeast lees consistently showed higher levels of S-volatiles: carbon disulfide and ethanethiol. These compounds were generally present at levels below their limit of detection, confirmed by compound dosing experiments in the control wine. However, some of the wine samples displayed the canned or overcooked vegetal notes characteristic of the proposed sulfur taint families. These observations suggest that conditions of the primary fermentation can impact the characters derived sur lie. Further, although odors characteristic of S-compounds were found in the wines, the compounds believed to be responsible for those odors were not present at high enough levels to generate the odor, suggesting more complex forces contribute to the off-odor formation sur lie.

Funding Support: American Vineyard Foundation and California Competitive Grant Program for Research in Viticulture and Enology.

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Evaluation of an Enhanced Modified Cash Still for the Determination of Volatile Acidity in Wine

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The addition of a Vigreux condenser as an enhancement to the commonly used Cash Volatile Acidity Still was tested to evaluate the reduction in lactic acid interference in the determination of volatile acidity in wine. Use of a modified Cash steam still for the determination of volatile acidity in wines is an AOAC standard method and is used by the TTB as a reference method. AOAC validation studies beginning in 1939 found that lactic acid carryover and several other factors could significantly affect results, but early researchers did not quantify these effects. We have previously determined that results reported using the modified Cash steam still (standard industrywide glassware) were frequently the sum of a series of interferences, with little relationship to the actual acetic acid levels in the samples. The addition of a Vigreux column was proposed over 60 years ago as means to remove lactic acid interference, but was neither tested nor adopted in the United States. Although the AOAC wine analysis committee considered it a worthy avenue to pursue, there was little interest because of the lack of widespread malolactic fermentation in the wines of that period. In a single laboratory validation of the reference method using the new enhanced glassware, we tested not only variables existing within the sample (such as acetic, lactic, succinic, sorbic, carbonic, and sulfurous acid, in addition to the actual pH of the sample) but also variables in performing the test (sample volume, distillate volume, condenser/distillate temperature, and boiling rate).

Funding support: Constellation Wines USA.

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Influence of Rootstocks on Growth, Yield, and Fruit Composition of Thompson Seedless Grown in India

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Thompson Seedless is the commercial cultivar grown for fresh grape and raisin consumption in India. Dog Ridge was the only rootstock used by grapegrowers before the late 1990s. However, Dog Ridge induced increased vigor to scions, resulting in reduced fruitfulness and fruit yield. To identify alternate rootstocks suitable for this cultivar, a study was initiated at National Research Center for Grapes during 2000. Treatments included own-rooted Thompson Seedless and Thompson Seedless grafted on Dog Ridge, 110R, 1103 P, 99R, and St. George rootstock. During initial years, Thompson Seedless grafted on Dog Ridge produced the highest yield with acceptable fruit quality. However, as the experiment progressed we observed uneven bud sprouting, blank areas on cordons, and reduced yield in vines grafted on Dog Ridge. Conversely, Thompson Seedless grafted on 110R performed well, with moderate vigor, increased fruitfulness, and consistently higher yield. Vines grafted on Dog Ridge and St. George produced lower yield owing to increased vigor measured in terms of pruning weight, total shoot length, and cane diameter. No significant influence of rootstock was observed for most fruit composition parameters. Berry size and appearance was improved by grafting on Dog Ridge and 110R rootstock. Own-rooted vines exhibited the smallest berry size. Based on the results of this study, the use of 110R rootstock was recommended for Thompson Seedless grapes.

Applying New Methods for Measuring Aboveground Vineyard Perennial Biomass

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Efforts to characterize the carbon footprint of viticulture are becoming common due to increasing interest in the role of various industries in the fate of carbon. Viticulture, like arboriculture, is the farming of perennial plantings that accumulate carbon in permanent biomass over one or more decades. In this project, aboveground vine perennial biomass was measured across a 20 acre vineyard with the intent of providing a spatially informed estimate of standing perennial biomass. Spatial variation in these values is illustrated with the use of remotely sensed and destructive harvest techniques. Data were collected on sampled vines using optical remote sensing, two forms of LiDAR remote sensing and ultimately using destructive harvest. Vine biomass samples ranged between 0.58 and 4.24 kg and were correlated among three nondestructive biomass estimation

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techniques. These values provide a basis for the estimation of the total biomass in the standing perennial wood. Although not part of this study, combining these data with commonly recorded harvest and pruning weights provides estimates of total above ground biomass as it fluctuates on an annual basis.

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Effects of Conventional and Mechanical Canopy Management on Performance of Cabernet Sauvignon

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This study was conducted to evaluate the effects of conventional and mechanical canopy management (CM) practices on canopy architecture, yield components, fruit composition, and their economic benefit on Cabernet Sauvignon/Freedom grapevines. The CM treatments included hand dormant-pruned (HP) vines retaining 32 to 40 buds per vine with no further CM (control); machine dormant-pruned vines to achieve an estimated 200% of the desired yield level then hand-pruned (MP+HP) to a density of 32 to 40 shoots per vine; and machine-pruned and shoot-thinned vines to achieve an estimated 130 to 140% of the desired yield when shoots were 10 to 20 cm and machine fruit-thinned (MP+MT). All vines were trained to a bilateral cordon with a foliage support wire on a two-wire vertical trellis. The number of count shoots per 30 cm of cordon was not affected. However, the number of noncount shoots per 30 cm of cordon for the MP+MT was 38% higher than the HP and the MP+HP. Regardless of CM method, the density of shoots per 30 cm of cordon was 26% and 32% higher at the head and the apical end of the cordon, respectively. The midday stem water potential of HP and MP+HP was 10% higher than MP+MT after veraison. Yield components were not affected by CM. There was no effect of CM on total soluble solids or juice pH. However, must anthocyanin of HP and MP+HP was 20%, 9% higher than MP+MT. The labor savings of MP+MT was 42% and 31% higher than HP and MP+HP, respectively. This study provides valuable information for growers that mechanical CM makes incremental adjustments for fine-tuning the canopy density and crop level a reality and is more cost and time effective.

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Understanding Wine Scores through Sensory Descriptive Analysis

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Eleven dry red wines from France, the United States, Chile, Argentina, Australia, and Portugal were selected based on their scores given by a wine magazine (higher than 90/100 points). With the price per bottle ranging from $40 to $192 and the scores from 90 to 96, no conclusive correlation between these two variables could be found. Since the critics evaluate the wines by appearance, odor, and flavor notes, we aimed to perform a PLSR (partial least squares regression) analysis to model the magazine scores for a given red wine based on sensory descriptive data (aroma/flavor, mouthfeel/taste, and color) generated by 22 trained judges. The strategy was three-fold: to create a groundwork by selecting and training a reliable panel that could discriminate among wines; to create a flavor/mouthfeel and color fingerprint of the wines by descriptive

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analysis (DA) and correspondence analysis (CA), respectively; and to use the sensory data (from DA and CA) to model the magazine scores by PLSR and understand which attributes are driving the scores. A full cross validation was carried out to estimate the prediction ability of the model for a new set of wines. Preliminary data shows that there are no significant differences between the obtained scores and the predicted scores, when performing a multiple pairwise comparison, at a Bonferroni corrected significance level of 0.0002.

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Effect of Canopy Management, Plastic Cover, and Calcium on Berry Splitting of the Grape cv. Flame Seedless

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Berry splitting reduces the quality of table grapes in Sonora, México, especially when high humidity is present after veraison. The objective of this study was to evaluate the effect of canopy management, polyethylene plastic cover on soil and calcium applications on berry splitting. The experiment was conducted in 2008, in La Costa de Hermosillo, Sonora. Canopy management consisted of positioning the shoots over the wires ("overwire") of the pergola and exposing the clusters to the direct applications of regulators and chemicals or leaving the shoots to fall down ("downward") and thus protecting the clusters from direct spraying of the chemicals. Black plastic covering was compared to the absence of the cover and calcium chloride (360 gr/ha) was applied three times during the application of gibberellic acid for sizing or was not applied. The treatments were distributed in a completely randomized block design with four replications and the experimental unit was a grapevine row with 20 plants. The percentage of clusters with more than four split berries was 3.75% for the overwire shoot treatment and 0.62% for the downward shoot treatment (significantly different, $p < 0.05$). Berry diameter was 1.95 cm for the overwire shoot treatment and significantly different ($p < 0.05$) from the downward shoot treatment (1.88 cm). Sugar content and berry color were not affected by the canopy management. Splitting, size, sugar content, and color of the berries were not affected by the plastic cover and chloride applications. Finally, there were no interactions among the factors in this study.

Polyethylene Plastic Tunnels Delay Budbreak of Table Grapes in the Sonoran Desert

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Table grapes produced in Sonora, Mexico, are mainly commercialized in May and June. To achieve this objective the plants are pruned and induced to break early in the season, which exposes shoots and inflorescences to temperatures below optimum for their growth. The aim of this work was to evaluate the effect of plastic covers on the soil and tunnels on budbreak and yield of the grape cv. Perlette. There were four treatments: polyethylene black plastic cover on the soil, polyethylene black plastic cover on the soil plus plastic tunnel, plastic tunnel, and control. The treatments were distributed in a completely randomized block design with three replications and the experimental unit was a row of seven plants. Grapevines were pruned on 19 Dec 2007 and applied with hydrogen cyanamide 2.5%. The plastic covers and tunnels were installed on 10 Jan 2008 and only the tunnels were removed on 5 Feb. Budbreak on 28 Jan under the plastic cover plus plastic tunnel and the plastic tunnel was 7 and 15%, respectively, which

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was lower than the control (100%) and the plastic cover (83%) ($p < 0.05$). Budbreak increased after the removal of the tunnels, and it was 85 and 95% under the plastic cover plus tunnel and the tunnel alone, respectively, with respect to the control, on 13 Feb. However, before thinning the number of clusters per plant were similar and were 27, 26, and 25 under the plastic cover plus tunnel, the tunnel alone, and the control, respectively ($p < 0.5$). Yield was not affected by the treatments.

Do Plant-Based Measurements of Vine Water Status Accurately Reflect Soil Moisture Content?

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A study was conducted during the 2008 season to examine the relation between soil moisture and plant-based measurements of vine water status while following an ET_c-based irrigation scheduling model. Two irrigation strategies were implemented in a Merlot vineyard in the Central Valley—a controlled deficit irrigation at 70% of crop evapotranspiration (ET_c) and a nondeficit treatment at 100% of ET_c—with both treatments applied during the entire growing season. Irrigation started in both treatments when vines reached -10 bars of midday leaf water potential. Soil moisture was assessed weekly by using watermark sensors installed at depths of 1, 2.5, and 4 feet. Midday leaf water potential and leaf stomatal conductance were also assessed at the same time using a pressure chamber and a leaf portable porometer. Soil moisture for the 100% ET_c treatment remained fairly constant during the season. However, soil moisture declined steadily for the 70% ET_c treatment until mid-August, and then increased gradually thereafter. The treatments maintained significant differences in midday leaf water potential and leaf-stomatal conductance for most of the season, but these parameters were more strongly linked to air temperature and vapor pressure deficit than to soil moisture. The results question the efficacy of using vine physiology-based parameters to schedule irrigation and suggest that a combined model, using a soil moisture threshold to apply the season's first irrigation and an ET_c-based irrigation schedule after that, might improve water use efficiency. This model will be tested during the 2009 season.

Analyzing Natural Diversity of Arbuscular Mycorrhizal Fungi in Conventional and Organic Vineyards of Central Chile

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The study and management of microbial interactions in the soil-plant interfaces play a key role in low-input-based viticulture. The objectives of this study were to study and characterize indigenous arbuscular mycorrhizal (AM) fungal communities in six conventional and organic vineyards in central Chile. The study was carried out between 2007 and 2008 at three localities: Cauquenes (35°54'S; 72°04'W), Sagrada Familia S (35°02'S; 71°16'W), and Chimbarongo (34°43'S; 71°02'W). Winegrape cultivars Cabernet Sauvignon, Chardonnay, País, Carménère, and Torontel were used. Rhizosphere soil and roots were sampled and analyzed in December 2007 and April and October 2008. Spores were extracted using centrifugation methods. Mycorrhizal root colonization and

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soil chemical analysis were conducted. None of the vineyards surveyed lacked spores, but genus composition differed among the vineyards. Most of the fungi were in the genus *Glomus*, *Acaulospora,* and *Scutellospora*. Spore diversity and quantity and mycorrhizal root colonization were greater in organic than in conventional vineyards.

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Evolution of the Color of Seeds during Ripening in Carménère Grapevines

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Carménère has high concentrations of phenols and methoxypyrazines; hence, winemakers must delay the harvest until this content declines in grapes. Sensory evaluation of grapes plays a key role to determine the harvest date in the later stages of berry ripening. During 2007–2008 season, the appearance evolution of Carménère seeds was evaluated as a harvest criterion and compared with the chemical and phenolic ripening. The samples were obtained from an organic vineyard located in Curicó Valley (Chile). After verasion, 100 berry seeds samples were collected weekly from medium vigor vines in order to register photographically the ventral and dorsal sides of each seed. Seed phenols, skin anthocyanins, and total phenols (determined by Glories method), titratable acidity, soluble solids, and pH were registered. A seed coat color chart with 12 colors was proposed for this cultivar. When the color number was higher than 10 (very dark brown), the soluble solids had reached 24 Brix one month previously, but skin anthocyanins and seed phenols presented a maximum and minimum, respectively. An inverse correlation between seed coat color and seed phenols was found from verasion until harvest time. This moment occurred 170 days postflowering, supporting the winemarkers' decision to wait for harvesting at the right time. Seed color can be a reliable, simple, and low-cost parameter to determine the correct ripeness of phenols in Carménère grapevines.

Grapevine Clones Selected in Chile

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Clonal selections of seven cultivars of *Vitis vinifera* were collected from the Central Valley of Chile. Plant identity and purity were determined by ampelographic and PCR-based molecular markers. Only virus-free vines were planted directly in the soil in COPEVAL S.A. Research Station located in Colchagua Valley at 1.0 x 2.0 m spacing and trained in unilateral Guyot. The vineyard system consisted of a randomized complete block design with three replications of 16 vines in each clone. Thirteen clones of cv. Cabernet Sauvignon, six of Carménère, four of Merlot, three of Cot, six of Pinot noir, five of Sauvignon vert, and two of Semillon were compared in yield, vegetative growth, vine phenology, berry composition at harvest, sensory characteristics, and chemical

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composition of wines. The results of statistical analysis during four seasons (2002–2006) identified the outstanding clones: Cabernet Sauvignon clone 060108 in yield, soluble solids, and anthocyanin content at harvest; Carménère clone 080309 in wine quality; Merlot clone 090104 in sensory attributes (fresh fruit); Cot clone 040213 in wine color; Pinot noir clone 100109 in wine quality; Sauvignon vert clone 010201 in early ripeness and wine quality. The cultivar Semillon clones did not show significant differences. Further research is needed in order to study these grapevine clones at an older stage.

Effects of Horticultural Oil on Carbon Assimilation and Fruit Set on Winegrapes

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The effects of horticultural oil on carbon assimilation and fruit set were examined. JMS Stylet oil was applied at trace bloom to Pinot gris grapevines in three consecutive growing seasons at 1% and 2% concentrations (v/v) plus a water control. Single leaf carbon assimilation (*A*) on leaves opposite basal clusters decreased the day after application at both application rates in each year, although the results varied among years. In year one, there were no differences in *A* between application rates. Both treatments returned to control rates of *A* within 12 days. In year two, there was a greater reduction in *A* at the 2% rate relative to the 1% rate. *A* in vines receiving the 2% concentration did not recover to control rates, while the vines receiving the 1% rate returned to control *A* rates within 16 days. In year three, the 2% application rate also reduced *A* more than the 1% rate. *A* in vines receiving the 1% concentration returned to control levels within nine days versus ≈four weeks at the 2% rate. Treated vines had reduced fruit set and, consequently, looser clusters in years one and two. There were no significant differences in fruit set in year three.

Production of SO_2 and SO_2 Binding Compounds by *Saccharomyces* during Alcoholic Fermentation and Impact on Wine Lactic Acid Bacteria

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Malolactic fermentation (MLF) is important in the production of wines and is commonly induced using commercial cultures. Successful MLF can be difficult because of yeast antagonism of the bacterium responsible for the secondary fermentation (*Oenococcus oeni*). In particular, yeast-produced SO_2 has been implicated in causing problematic MLF. SO_2 is commonly bound to acetaldehyde, pyruvic acid, and keto-glutaric acid in wine, compounds produced by yeast. However, little is known regarding the toxicity of the different forms of bound SO_2. Therefore, the purpose of this research was to investigate the production of SO_2, acetaldehyde, pyruvic acid, and keto-glutaric acid by various commercial wine yeast strains and the impact these have on the MLF. Fermen-

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tations were conducted in a synthetic grape juice where viable yeast cell growth, SO_2, acetaldehyde, pyruvic acid, and keto-glutaric acid were measured during the alcoholic fermentation. At weekly intervals 100 mL samples were taken from the fermentations, sterile-filtered to remove the yeast, and inoculated with *O. oeni* to induce MLF. MLF was monitored by measuring malic acid and bacterial viable cell counts. All of the 12 yeast strains tested produced different amounts of SO_2, acetaldehyde, pyruvic acid, and keto-glutaric acid. Some yeast strains produced significantly more SO_2, and *O. oeni* viability decreased rapidly when inoculated into these wines. Very little if any free SO_2 was measured, indicating that bound SO_2 was responsible for the inhibition. In fall 2008, fermentations conducted in a Pinot gris grape juice confirmed the findings from the synthetic grape juice model system. Yeast produced varying amounts of SO_2, acetaldehyde, pyruvic acid, and keto-glutaric acid and MLF was inhibited in wines containing the highest concentration of SO_2. As yet, there appears to be no clear interaction between the amount of acetaldehyde, pyruvic acid, or keto-glutaric acid produced by the yeast and bacterial inhibition.

Yeast Population Dynamics in Natural and Inoculated Sweet Wine Fermentations

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The Erbaluce di Caluso sweet wine is produced in Piedmont (Italy) from grapes of the homonymous variety that are naturally dried. Alcoholic fermentation is carried out by autochthonous yeasts or inoculated starters. In the present study we compared the yeast population dynamics and the evolution of the main chemical components in a natural and an inoculated fermentation. We used culture-dependent and independent molecular approaches to describe the yeast populations. As expected, in the case of the inoculated fermentation, *Saccharomyces cerevisiae* was the main population throughout the course of the investigation. On the other hand, increased biodiversity was detected in the natural fermentation. After the initial days of fermentation, characterized by the presence of different mold species and of *Candida zemplinina*, which also persisted later in the fermentation, at the DNA level we mainly detected *S. cerevisiae*. When RNA, an indicator of viability and possibly metabolic activity, was taken into consideration, *Torulaspora delbrueckii* and *C. zemplinina* were also detected. Surprisingly, by culture-dependent methods *T. delbrueckii* was not detected while *Kluyveromyces thermotolerans* and *S. cerevisiae*, together with *Hanseniaspora uvarum* and *Metschnikowia fructicola*, were the main populations identified. From the chemical analysis performed, it can be concluded that the natural fermentation, in terms of sugars consumption as well as alcohol and glycerol production, was superior to the inoculated. This work underlines the importance and perhaps the superiority of the autochthonous microflora in conducting the alcoholic fermentation for the production of this wine.

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Comparing Conventional and Modified Methods for Initiating Malolactic Fermentations in Apple Cider

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Malolactic fermentation is a valuable deacidification technique that can be used to increase the quality of hard apple cider. The characteristics of the organism used to initiate the fermentation along with other factors such as sulfur dioxide and ethanol can affect the efficiency of the fermentation. Conventional fermentation methods consist of inoculating apple cider with the yeast *Saccharomyces cerevisiae* Montrachet to initiate the primary fermentation followed by inoculation with the lactic acid bacterium *Oenococcus oeni* MCW to initiate the malolactic fermentation. This method was compared to a modified method using a genetically modified yeast, *Saccharomyces cerevisiae* ML01, which has been engineered for wine production and performs both the primary fermentation and malolactic fermentation simultaneously without the addition of lactic acid bacteria. The effect of sulfur dioxide (50 and 80 ppm) and ethanol (6, 7, 9, and 11% [v/v]) on the efficiency of the malolactic fermentations using both fermentation methods was also evaluated. Parameters monitored during the fermentation included organic acid content, titratable acidity, color, pH, ethanol production, and sugar utilization. There was no effect of sulfur dioxide or ethanol on the fermentations initiated by *S. cerevisiae* ML01. *Saccharomyces cerevisiae* ML01 was also able to complete the primary fermentation and malolactic fermentation simultaneously, thus increasing efficiency by shortening the fermentation time. Sulfur dioxide had no effect on the malolactic fermentation initiated by *O. oeni* MCW. However, as ethanol levels increased the malolactic fermentation was inhibited.

Effect of Ethanol and Glucose on Aroma Compound Partitioning between the Headspace and Wine Matrix

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This study investigates how wine macromolecules contribute to partitioning of potent volatile compounds into the headspace. A headspace solid-phase microextraction (HS-SPME) method was optimized to establish equilibrium between the SPME fiber and the headspace above a model wine solution, thus reflecting the "true" headspace concentration. This optimized method was then used to study the influence of ethanol and glucose at varied concentrations that would be found in an actual juice or wine on the release of 20 volatile compounds considered to play a role in wine aroma. Varied concentrations of ethanol showed significant differences for all volatile compounds with the exception of limonene; however, there was no significant influence of varied glucose concentrations. Two white and two red wines were analyzed to observe the effect of diluting the wines with water and ethanol. Responses were similar to that observed in

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the model experiments. Analytes from the wines were grouped using hierarchal clustering and analyzed using a one-way analysis of variance to determine the significance of dilution compared to modification of ethanol concentration. The results of this study identify ethanol and glucose as having a significant influence on volatile compound partitioning between the liquid matrix and the headspace. These results also help to explain sensory research observations that indicate that ethanol can suppress the fruit aroma attributes in wine. Further sensory analysis is warranted to better understand the influence of ethanol on aroma perception given the gradual shift toward higher alcohol wines in recent years.

Comparison of Combined Titrametric Analysis of TA and YAN with Traditional Measurements

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Yeast assimilable nitrogen (YAN) by Formol analysis was combined with titratable acidity (TA) into a two-part procedure using commercial white and red grape juice. Initially, TA was determined by direct titration of a 5.0 mL juice sample to pH 8.2 in a 30-mL beaker. Subsequently, 2.0 mL of formaldehyde (pH 8.2–8.4) was added to the sample, which was then titrated to pH 8.2. The corresponding volumes of standardized NaOH were then used to calculate TA and YAN. Results using combined TA/Formol analysis were compared with corresponding results using TA and Formol titrations separately. TA results for combined methods using white juice (n = 10) and red juice (n = 10) were 5.7 g/L ± 0.1 and 6.5 g/L ± 0.1, respectively. YAN results for the same two lots were 209 mg/L ± 1 and 240 mg/L ± 13. By comparison, TA and YAN for the same juices analyzed separately yielded TA and YAN values of 5.6 g/L ± 0.1 and 6.4 g/L ± 0.1 and 204 mg/L ± 9 and 234 mg/L ± 9. Given the anticipated repeatability of these analytical methods in a practical winery situation, there are no significant differences in the results produced by the combined procedure, compared to the traditional procedures. There are some significant savings in operational time.

Inhibitory Effect of Chitinases Isolated from Semillon Grapes on Growth of Grapevine Pathogens

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We investigated the characteristics and antifungal activity of chitinases isolated from Semillon grapes. Chitinase proteins were effectively isolated from the juice of Semillon grapes by chitin affinity chromatography. Native and SDS-PAGE analyses of the fraction showing chitin affinity demonstrated only the presence of protein bands from chitinases. The fraction showing chitin affinity contained three types of class IV chitinases with isoelectric points of 4.73, 4.60, and 7.87. These chitinases actively hydrolyzed chitin under acidic conditions (pH 4.0 to 4.5). The active fraction was found to inhibit myce-

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lial growth of *Botrytis cinerea*; the inhibitory effect was due to the activity of chitinases. However, when the active fraction was tested on *Glomerella cingulata*, the growth inhibition observed was markedly less than that seen on *B. cinerea*. The synergistic effect of chitinases and sugars (glucose and fructose) was observed clearly in media containing greater than 20% sugars, but not in media with less than 15% sugar. Twenty strains of *B. cinerea* were collected from the experimental vineyard and examined for sensitivity to the fraction showing chitin affinity. This fraction inhibited the growth of all 20 strains.

Impact of Basal Leaf Removal on Methoxypyrazine Concentration in Cabernet franc and Merlot Grapes

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The methoxypyrazines are a class of potent aroma compounds associated with the green, bell pepper aroma of Cabernet franc, Merlot, and other Bordeaux winegrape cultivars. Because vinification and cellaring practices cannot selectively remove methoxypyrazines from wine, control should begin in the vineyard. Field studies were conducted on Cabernet franc and Merlot to evaluate the effects of basal leaf removal timing and severity on methoxypyrazine concentration in grape berries. Treatments consisted of removing either 3 or 5 leaves from the first five nodes from each shoot at berry set, 30 days after berry set, and 50 days after berry set. Berry samples were taken 5 days after each treatment timing and at harvest. In 2007, all leaf removal treatments significantly reduced methoxypyrazine concentrations (47 to 88%) in mature Cabernet franc berries. In 2008, no significant differences in methoxypyrazine concentration were observed across treatments in mature Cabernet franc berries, and all leaf removal treatments significantly reduced methoxypyrazine concentrations (38 to 52%) in mature Merlot berries. Reductions in methoxypyrazine concentrations were also observed among treatments before veraison. Results suggest that early season basal leaf removal could be an effective management strategy to control methoxypyrazine concentration in grape berries.

Evaluation of a New Tool to Aid in Viticultural Decision Making Based on Desired Wine Characteristics

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There are a multitude of vineyard practices that can be manipulated in order to affect changes in the final chemical and sensory characteristics of a wine. Given the complex relationship between viticultural practices and wine characteristics, it is often difficult to predict the outcome and optimize growing conditions for key target attributes in wine (e.g., specific aromas, flavors, or phenolic profiles). Neural networks can be used as a tool for evaluating complex nonlinear relationships among a high number of input variables. For this reason, neural network methodologies have been used to model viticultural effects on the chemical profile of wines. In any viticulture trial, a long-term

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validation process is needed in order to evaluate the efficacy of any technique. Data collected from the 2000–2003 vintages in experimental vineyards in Oakville were used in this study to identify combinations of viticultural practices that have the most impact on the chemical characteristics of these Cabernet Sauvignon wines. The identified combinations of practices, together with data from the associated wines, were used to train neural network models. In 2008, Cabernet Sauvignon grapes were grown with five different viticultural treatments. These were subsequently made into wine in order to examine how well our models predicted the outcome of new combinations of viticultural practices. Treatments were purposely chosen to represent a wide range of predicted chemical profiles for these wines.

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Detection of Volatile Compounds Released in Vine Canopies for Use in Field Measures of Fruit Maturity

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Plants communicate using visual and aromatic signals. Colors and aromas in flowers and fruits attract pollinators and animals for seed dispersal, while other volatile signals report stress from biotic and abiotic sources. Despite our understanding of the strong correlation between plant status and volatile compound emissions, little work has been performed to investigate the identity of such compounds in *Vitis vinifera*. By measuring and identifying compounds present in vineyards and correlating them to vine or fruit states (healthy, diseased, underripe, mature, water stressed, etc.), we intend to discover key marker compounds that could inform viticulturists about the status of a particular vine or vineyard and, more specifically, about the best time points for harvest. To date, methods have been established for trapping canopy volatiles in the vineyard and the subsequent analysis of these complex mixtures in the lab using gas chromatography-coupled time-of-flight mass spectrometry (GC-TOF-MS). Ongoing work includes database development for chromatogram annotation and volatile compound tracking. Initial analysis of data collected during the 2008 harvest season in three Cabernet Sauvignon blocks has identified correlations between volatile compounds captured in the canopies and berry Brix, pH, and TA values. More refined analysis is currently in progress to identify these compounds.

Cytonomics: An Innovative Fermentation Control System Based on Flow Cytometry

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The aim of this application study was to present methods that provide deeper insight into yeast life and can therefore be used as a fermentation control system. The whole

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process control combines conventional data and physiological measurements using flow cytometry. The methods were practically tested in a brewery, as beer is produced throughout the year and fermentation time tends to be shorter. The measurements, which can be combined depending on the goal of research, are cell size and cell shape, cell cycle, viability, stored glycogen, trehalose content, intracellular neutral lipids, protease activity, apoptosis, number of bud scars, mitochondria activity, oxygen radicals, membrane potential, intracellular pH value, and biological contamination of the media. Considering all the different results, the system provides comprehensive information about the yeast cell using only one instrument. In every stage of fermentation from inoculation to cell death, monitoring viability and functionality parameters allows the operator to find the best inoculation time and to see stress reactions before fermentation problems occur. This might help to guarantee best possible viability of the culture and prevent sluggish or stuck fermentation. Even the progression of cell death and autolysis and the reasons for that can be monitored with a selection of the available parameters. If applied correctly, flow cytometry is an extremely flexible tool to analyze intracellular activity and metabolite inside yeast cells. Its use as a yeast control instrument in research and industry might help to better understand and influence the fermentation process.

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Rootstock-Scion Interactions and Correlations between Vine Vigor and Grape Quality

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A good rootstock-scion combination is necessary for optimum vine growth and fruit quality in each region. Experiments were conducted at the experimental vineyards of the University of Pannonia Georgikon Faculty of Agriculture in Cserszegtomaj (46°49'45"N; 17°15'16"E) using *Vitis vinifera* cvs. Kékfrankos and Cabernet Sauvignon. These were grafted on the following rootstock: Teleki Fuhr SO4, Fercal, Georgikon 28, Teleki-Kober 5BB, and Teleki 5C. The main effect of the rootstock was manifested in the vegetative vigor and yields of the vines. Rootstock impacts on soluble solids and acid content of berries was much more limited. The experiment design consisted of five 8-vine replicates of each rootstock-scion combination planted in 2004 using 2.9 x 1 m (row x vine) spacing. The vines were trained to a single cordon at 0.6 m and balance-pruned. The following data was taken: pruning weight, crop yield, yield/pruning weight ratio, and juice soluble solid and acid content. Regressions were calculated between the acid and soluble solid values versus yield/pruning weight ratios. The rootstocks had an impact on each parameter. The greatest impact was on yield/pruning weight ratios and crop yields. The yields differed by 30 and 33.5% between the lowest and highest production with Kékfrankos and Cabernet Sauvignon, respectively. The volume of the grape berry skin was also influenced by rootstocks and correlated with color intensity.

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Inheritance of *Xiphinema index* Resistance Derived from *Vitis arizonica*

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Resistance to *Xiphinema index*, the dagger nematode vector of grapevine fanleaf virus (GFLV), is key to the development of rootstocks capable of resisting fanleaf degeneration. A breeding program to develop rootstock with resistance to fanleaf has been underway for many years at UC Davis. In 2007 controlled crosses were made to study the inheritance of *X. index* resistance, originating from b40-14, a form of *Vitis arizonica* from Loreto, Baja California. Previous studies found that *X. index* resistance is controlled by a single homozygous resistant locus. Seeds from these crosses were germinated and grown in a greenhouse. The DNA of all plants was screened with SSR markers to eliminate off-types. In total, 296 plants from three populations (0701, 0704, and 0705) were transferred to the field in 2008. These plants were used to create subsets of four replicates, which were inoculated with *X. index*-infested soil under greenhouse conditions. Positive and negative controls, as well as the parent plants, were included

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in this greenhouse study. After four to five weeks in the infested soils, the roots of each population were evaluated for galling. Leaf tissue samples were harvested and DNA was extracted in preparation of developing an SSR-based marker genetic linkage map so that SSR markers linked to this excellent source of *X. index* resistance could be used in marker-assisted selection to expedite rootstock breeding.

Funding support: South African Table Grape Industry, California Grape Rootstock Improvement Commission, California Grapevine Rootstock Research Foundation, CDFA Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board, California Table Grape Commission, and Louis P. Martini Endowed Chair funds.

Evaluation of the Susceptibility of Rootstock Varieties Against *Phomopsis viticola* (Sacc.)

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One of the most important factors for economically successful viticulture is to have a healthy, complete vineyard without missing plants. However, vines frequently die before they have reached the end of the vineyard's lifespan. In Hungary, a survey found that about half of the vineyards had greater than 6% of vines missing. These losses are often due to vine decline caused by a group of parasitic fungi that attach to the vine's xylem tissue. More than 80 species of fungi have been listed as responsible casual agents of vine decline in the international literature. However, very little information is available regarding the types of fungi involved in the vine decline of grape rootstocks. Rootstock mothervines are regarded as one of the important sources of vine decline in vineyards. Thus, our goals were to compare the susceptibility of currently used grape rootstock varieties and to test the susceptibility of newly bred Hungarian rootstock selections. The rootstocks originated from the experimental plot of the University of Pannonia Georgikon Faculty, in Cserszegtomaj (46°48'22"N; 17°13'52"E). Twenty different varieties were tested by inoculating with the fungus *Phomopsis viticola* (Sacc.). At the end of the experiment the degree of the tissue discoloration was measured, and the growth rate of the varieties was compared. We were able to reisolate the fungus from 76% of the inoculated vines, and the length and degree of discoloration varied widely. These results can help growers avoid rootstocks that are susceptible to vine decline fungi. They also suggest that it will be possible to breed a rootstock variety that is resistant to vine decline pathogens.

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Effect of Simulated Shipping Conditions on Sensory Attributes and Volatile Compounds in Six Wines

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A major concern in shipping wine is receiving wine at its destination in the same condition as it left. In this study, the effects of shipping conditions on six varietal wines were explored. Four white wines and four red wines were exposed to four different storage conditions to create 32 treatments. The white wines used in the study were from one producer and of the same vintage, and included Riesling, Chardonnay, Gewürztraminer, and Sauvignon blanc. The red wines included two Merlot and two Cabernet Sauvignon. Storage conditions were 20°C, 40°C, 20/40°C (reflecting diurnal variation in temperatures), and a sample which traveled in the trunk of a car, for three weeks. The 32 wines were evaluated using sensory descriptive analysis. Twelve judges for whites and 14 judges for reds rated the wines for 14 and 19 attributes, respectively. Volatiles were analyzed using HS-SPME GC-MS. Both the sensory and analytical results of the study showed significant differences between the wines stored at the higher temperature conditions. Differences were noted for a number of compounds including TDN and vitispirane, which are both characteristic of aged wines. This is the first study that has assessed sensory changes in wines under conditions that could be experienced by a wine in transit.

Effect of Pectinase and Extended Maceration on the Extraction of Tannins in Norton Wine

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Wines made from Norton grapes tend to be deficient in tannin while having high anthocyanin levels. There is an interest by winemakers in optimizing tannin extraction during the production of these wines. Tannin is needed in red wines as it improves color stability by the formation of polymeric pigments comprised of tannin and anthocyanin molecules. Tannin, within limits, can also have a positive impact on the sensory characteristics of a wine. Two enological procedures (addition of pectinase and extended maceration) were evaluated for their impact on tannin and anthocyanin levels in Norton wine. A control treatment without enzyme addition was compared with two treatments having the same level of pectinase added. The control treatment and one of the enzyme treatments had a maceration period of one week (during fermentation). The other enzyme treatment had an extended maceration period of three weeks after the initial one-week fermentation. The use of pectinase resulted in wines with significantly higher levels of tannin, polymeric pigment (small, large, and total), total red pigment, total phenolics, and color (absorbance at 420, 520, and 620 nm). Extended

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maceration resulted in significant reductions in all of these parameters. The increases in phenolic compounds observed when using pectinase were lost during the course of the three-week extended maceration. Tannin, total red pigment, and total phenolic levels peaked in all of the wines three days after the start of the fermentations. Early pressing improved tannin retention.

Nitrogen and Yeast Strain Effects on Low Molecular Weight Alcohol Production

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Volatile, low molecular weight alcohols are produced during yeast fermentation. They include straight-chain, branched-chain, and cyclic molecules. Methanol is present in grape must and is not affected by fermentation. Low molecular weight alcohols affect wine quality and are precursors to esters. Ester productivity is an inherent characteristic of yeast, but low molecular weight alcohol production is not necessarily yeast strain dependent. Nitrogen availability affects the development of both compounds; high levels of must nitrogen produce wines high in esters but low in volatile alcohols. Furthermore, some amino acids are direct precursors to low molecular weight alcohols. The present study used a gas chromatographic method coupled with headspace solid-phase microextraction (HS-SPME-GC) to evaluate the production of low molecular weight alcohols by three yeast strains of varying ester productivity. A nitrogen poor Chardonnay must was used; additions of nitrogen from DAP or amino acids were tested. Eight alcohols, one ester, and nitrogen utilization were monitored. The yeast strain affected 2-methyl-1-butanol, 1-propanol, and ethyl acetate concentrations. The high ester producing yeast evolved higher levels of 1-propanol and ethyl acetate, but not 2-methyl-1-butanol. Nitrogen supplementation affected 2-methyl-1-butanol and phenylethanol concentrations. Utilization of amino acid precursors increased upon the addition of either form of nitrogen. This research indicates that adding a moderate amount of nitrogen to a nitrogen-poor must does not significantly affect the production of low molecular weight alcohols. Furthermore, ester productivity does not significantly affect a yeast strain's ability to produce low molecular weight alcohols.

Funding support: American Vineyard Foundation and the California Competitive Grants Program for Research in Viticulture and Enology.

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Effect of Seaweed Extract on Cluster Architecture and Set of Pinot noir Winegrapes

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A three-year trial, initiated in 2006 on Pinot noir wine grapes in California's Central Coast was implemented to test the hypothesis that applications of seaweed extract will increase set and improve cluster architecture. This vineyard has historically had a problem with shatter, the loss of berries shortly after bloom. Because of soil applications, a strip design was used, and the same vines received similar treatments each year. Treated clusters reached a greater percentage of their final weight earlier in the season than the control. More uniformity in ripening was also exhibited with seaweed treatment. In each year, cluster length was increased 8 to 15% at veraison. Set was increased 9 to 22%. No significant differences in pH, Brix, or titratable acidity were detected between treatments. Composite juice samples indicate little to no differences in juice quality. These results indicate that Acadian LSC is a viable option to help reduce shatter, increase rachis stretch, and overall yield, grape, and bunch uniformity, in Pinot noir winegrapes, while still producing quality juice.

Permeation of Taint Compounds through Different Closures in Wine Bottles

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The oxygen barrier properties of closures play an important role in wine development after bottling, but also on its protection against the external environment. This presentation outlines the results from an ongoing investigation to find evidence that the different oxygen barrier properties of closures affect the permeation of exogenous compounds when bottled wine is stored in a contaminated environment. The study was conducted to determine whether "taint" compounds from external environment could migrate through different commercial closures and contaminate wines after bottling. The bottled wine was sealed with a natural cork stopper, a microagglomerate cork stopper, and a synthetic closure and stored under contaminated environment. The storage atmosphere was artificially contaminated with two deuterium-labeled compounds, (d_5)-2,4,6-trichloroanisole (TCA) and ($13C_6$)-2,4,6-trichlorophenol (TCP). Wines and closure fractions (outer, middle, and inner) were assessed over time for the concentration of (d_5)-2,4,6-trichloroanisol and ($13C_6$)-2,4,6-trichlorophenol by SPME-GC-MS. The results collected throughout the first 12 months of experiment showed strong correlations between the oxygen barrier properties of closures and the migration of deuterium compounds. Under the conditions of this experiment, the natural and microagglomerate cork stoppers were effective barriers to the transmission of exogenous contaminants. Conversely, (d_5)-TCA and ($13C_6$)-TCP penetrated throughout synthetic closures and contaminated the wine.

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Dry Soak Sensory Screening for Large-Format Corks and Its Correlation to Releasable TCA Analysis

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A sensory evaluation method has been implemented to ensure that most tested corks will not compromise the wine that these closures protect inside the glass bottle. This method was named "dry soak screening." Large-format natural corks were assessed for TCA taint and other noncharacteristic aromas by individually placing them with a few drops of water inside sealed glass jars. The closures were "sniffed" by an expert panel after 24-hr equilibration. From a population of 2,296 corks, 138 specimens (6% of total population) were retained due to resenting odors, ranging from mild to severe. All retained corks were analyzed for releasable TCA by SPME GC-MS. Results indicated that 95 retained corks had concentrations of 1.0 ppt or less. Most of these corks had very mild noncharacteristic aromas and very few TCA sensory remarks. Thirty retained corks (1.3%) had concentrations between 1.0 and 5.0 ppt. Most of these corks had nontypical TCA aromas described as ashtray, musty, moldy, dirty, and wet cardboard. Thirteen retained corks (0.56%) had concentrations higher than 5.0 ppt and mostly displayed the typical TCA aroma. The number of TCA sensory remarks correlated fairly well with the analytical results. Other unusual noncharacteristic aromas were also tallied and explained. Dry soak screening has been used by our organization with great success. It is a clean, rapid, and, most importantly, nondestructive method ideal for the identification of off-aromas in individual large-format natural corks.

Solid-Phase Extraction Sample Preparation Application for Low Concentration Wine Compounds

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Use of pesticides in agriculture is widespread and pesticides are prevalent in the environment. Pesticide residues can contaminate soil and water sources from many miles distance from the source. Phenoxy acid herbicides (2,4-dichlorophenoxy-acetic acid) have been widely used for a variety of applications. Their affect on human health is still being debated even though they have been in use for decades. There is a need for quick, simple, and easy to perform analysis methods of wine to ensure that the consumer is not exposed to harmful amounts of this type of pesticide. Solid-phase extraction (SPE) is one of the most widely used methods for concentration and sample purification during pesticide residue analysis. The wine matrix can be difficult to work with when using traditional reverse-phase sorbents for SPE methods because the presence of alcohol in the samples can result in the decreased retention of many analytes. Empore disks work very well for liquid samples, and different varieties of sorbent activities can be used to selectively isolate specific classes of compounds. The presented method for the analysis of pesticide residue in wine is simple, fast, and uses minimal amounts of solvent and readily available equipment.

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Effects of Postbottling Oxygen Management on Chardonnay Wines

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The purpose of this study was to measure the chemical changes in a Chardonnay wine over time that could be influenced by oxygen management postbottling. The availability of oxygen to a wine after bottling can have both beneficial and detrimental effects over time. A Chardonnay wine was divided into four treatments: aged in stainless steel with and without lees and aged in oak barrels with and without lees. Wines from each of the four treatments after 6 months of aging were then bottled in 375-mL bottles. The bottles were closed with Nomacorc closures and stored vertically at about 15°C. Four different closures were used with different oxygen transfer rates. The combinations of aging and oxygen storage conditions produced 16 different wines. Dissolved oxygen in the wine and in the headspace of the bottles was measured with PreSens technology over time for each of the 16 wines, providing dynamic monitoring of consumed oxygen. Total/free SO_2, % EtOH, pH, 280 and 420 nm, phenols, esters, acetaldehyde, and organic acids were measured periodically over 10 months.

Funding support: Nomacorc.

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Sensory Evaluation of Postbottling Oxygen Management on Chardonnay Wines

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The effects of postbottling oxygen management on the sensory characteristics of a Chardonnay wine were explored. Postfermentation, a Chardonnay wine was aged using a combination of either stainless steel or oak barrels, and with or without lees contact to give four wines. After 6 months of aging the four wines were bottled into 375-mL bottles. The bottles were closed with Nomacorc closures and then submitted to four different storage conditions each with a different level of oxygen availability. The combination of aging and storage conditions produced the 16 wines. Using standard sensory methods the 16 wines were rated for 13 attributes by 14 judges in triplicate. The results from the sensory analysis were analyzed using principal component analysis (PCA) and means separation. Eight of the 13 attributes were determined as significant to discriminate the 16 wines. The PCA explained 96% of the variation within the first two components. The first component accounted for 81% of the variation and separated the wines in the positive direction by vanilla and oak aroma and in the negative direction by citrus and fresh vegetative aroma. The second component accounted for 15% of the variation and was driven by oxidative aroma in the positive direction. This sensory evaluation was performed at the conclusion of a 10-month chemical study. Partial least squares and multifactor analysis were used to show relationships between the sensory and chemistry of the 16 wines.

Funding support: Nomacorc.

Inert Atmosphere Grape Processing Kappa Neutral and Neutral 2 for High-Quality White Wines

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White wine production in an atmosphere without oxygen has until now considered only the protection of juice from the pressing phase to bottling. Diemme's Neutral Line now allows the creation and the preservation of an inert atmosphere during all phases before fermentation, avoiding oxidation starting with the breaking of the berry and protecting sensitive products from oxidation in all processing phases. The coupling of the no-oxygen Kappa Neutral destemmer-crusher and membrane press Neutral 2 system results in juices that preserve the intrinsic sensory features of the grapes and carry over to the finished wines. In juices obtained with Neutral technology, we found dissolved oxygen values at the Kappa Neutral destemmer-crusher outlet from 4 to 6 times lower than those obtained using a normal destemmer-crusher. The Neutral 2 membrane press system yields free run and pressed juice with minimal oxygen contact. The Neutral technology for destemmer-crushers and membrane presses opens up interesting prospects

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in the phase before fermentation for processing grapes especially rich in polyphenolic substances and for the production of high-quality aromatic wines. System Neutral processed wines obtained from Verdicchio grapes harvested at the beginning and at the end of the 2008 harvest have been particularly appreciated.

Increasing Winery Eco-Efficiency with a Natural Alternative for Tartrate Stabilization

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Research by DSM Food Specialties has lead to greater understanding of the biochemistry of mannoproteins and their effects on tartrate stabilization of wine. Although mannoproteins are natural components of yeast and therefore naturally present in wines, they represent a wide diversity of molecules. DSM has identified the optimal mannoprotein fraction for tartrate stabilization and developed a process for its selective extraction from yeast. These mannoproteins available under the brand name Claristar are a natural alternative for the currently used technologies for preventing the apparition of crystals in wine bottles. Effective at preventing tartrate precipitation without impacting wine turbidity, sensory attributes, or composition, these specific liquid mannoproteins, in addition to their performances and ease of use, have a superior eco-efficiency over other tartrate stabilization alternatives: cold stabilization and electrodialysis. The use of these specific mannoproteins eliminates the water and energy usage in tartrate stabilization at the winery. Moreover, in comparison to existing solutions for tartrate stabilization, Claristar significantly reduces the ecological footprint from the perspective of the value chain as a whole: water use is reduced with 26 to 52% and energy use and carbon footprint with 45 to 70%, depending on the scale and efficiency of the winery. The emissions to water are reduced with 65%. An eco-efficient solution for tartrate stabilization fits with increasing market sensitivity to sustainability in the wine industry.

Smoke Taint in Grapes and Wines

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The California wildfires of 2008, with over 1,700 individual fires at their peak, exposed vines to smoke in various viticulture areas. Smoke compounds absorbed by vines can cause an objectionable character in finished wines known as "smoke taint." In Australia, two volatile phenols, guaiacol and 4-methylguaiacol, have been identified as useful markers for smoke taint. With hundreds of grape samples and over 1,000 wines analyzed, data obtained from California's harvest 2008 provided new information and confirmed many of the observations reported elsewhere. Levels of guaiacol in grapes not exposed to smoke seldom exceeded 0.3 g/kg. Samples from areas exposed to heavy smoke were found as high as 55 g/kg. The guaiacol/4-methylguaiacol ratio averaged 3.7:1. Levels in juice obtained from freshly pressed smoke-exposed berries were lower than levels measured in ground whole berries, confirming that a significant part of smoke compounds is located in skins. Maceration of popped berries further increased

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measured smoke markers. As reported in Australia, it appears that smoke taint compounds may be liberated from nonvolatile precursors. Guaiacol concentrations increased during the alcoholic fermentation and were typically three times higher in white/rose wines than in juice before fermentation. With red wines, final levels were approximately five times higher than originally measured in corresponding grapes. Smoke taint could be identified sensorially in wines with guaiacol levels as low as 5 g/L, but sulfides appeared to possess a strong masking effect. Trials regarding the removal of smoke compounds from wines are currently being conducted.

Flavonol Precipitates in Sangiovese Wines

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Quercetin, a flavonol aglycone, is the most common precipitate found in commercial bottling of California Sangiovese. Flavonol glycosides are present in high concentrations in Sangiovese grape skins and are extracted during fermentation. Cleavage of the glycoside moiety occurs during fermentation, barrel storage, and bottle aging. The resulting aglycone is much less soluble in wine than the glycosides. Flavonol aglycone solubility is increased due to the formation of protective colloids formed with anthocyanins and tannins. In the case of quercetin, solubility can be increased more than 10-fold by addition of anthocyanins in model wines. The interaction appears to be related to copigmentation, as red color of the anthocyanin-containing model wines was enhanced by the quercetin addition. Postbottling, quercetin glycosides continue to hydrolyze, increasing aglycone concentrations. Simultaneously, anthocyanin concentrations decrease. In a Sangiovese wine aging study, quercetin increased from 14 to 33 mg/L while anthocyanins decreased from 320 to 26 mg/L over five years. When the aglycone concentration exceeds solubility limits, hairlike yellowish quercetin crystals form which combine to form gelatinous lumps. Quercetin is often supersaturated in Sangiovese wine and seeding with powdered quercetin can result in a 20% reduction of quercetin concentrations in solution. Sangiovese appears to be particularly prone to quercetin precipitates due to exceptionally high flavonol concentrations along with low concentrations of anthocyanins and tannins.

A Novel Screwcap Liner with Variable OTR and the Effects of Its Components on OTR

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A novel liner for roll-on tamper-evident (ROTE) screwcaps is being developed for the wine industry. This liner will allow the winemaker to choose a ROTE cap that provides an optimal oxygen transmission rate (OTR) for their product. Data from design-of-experiment (DOE) trials gives resulting predictive equations. The goal of this work is to provide the wine industry with screwcaps having OTRs specifically targeted for the needs of various wines. Currently there are only two cap liners widely used in the wine industry. One liner employs saran and the other tin foil as the primary oxygen barrier. To develop baseline data, Mocon Oxtran tests were conducted to determine the OTR of

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packages finished with natural cork and ROTE caps utilizing these two lining materials. The natural cork finished package—stored inverted—had an OTR of ≈0.0005 cc O2/day, the saran-lined cap had an OTR of ≈0.001 cc O2/day, and the foil-lined cap had an OTR of 0.00001 cc O2/day. By properly selecting and physically modifying the components of the novel liner, cap-finished package OTRs can be made to match that of a quality natural cork. The OTRs can also be adjusted to range anywhere between the saran- and foil-lined caps. Mathematical models developed using the DOE approach will allow the prediction of OTRs based on the liner design, bottle characteristics, and capper set-up.

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Comparison of a Leaf Porometer and a Pressure Chamber for Assessing Vine Water Status in Chardonnay and Zinfandel Grapes

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Our work in progress compares a leaf porometer (Decagon Devices) and a pressure chamber (PMS Instruments) for assessing vine water status in Chardonnay and Zinfandel winegrapes in the Paso Robles area of California. Treatments were both varietals watered at 100%, 50%, and 0% of amounts recommended by soil moisture monitoring sites located near the plots. The plots consisted of five vines where there were two 0.5 gph emitters (100%), one 0.5 gph emitter (50%), or no emitter (0%). Treatments were initiated on 1 July 2008 and continued through harvest. Measurement of stomatal resistance was made by selecting the most recently mature fully expanded sunlight leaf along a cane and placing the sensor head immediately to the left or right of the petiole-blade attachment point. The sensor was attached for 30 sec, at which time the stomatal resistance was recorded. This same leaf was then removed and used to determine leaf water potential in the pressure chamber using standard techniques. Simple linear regression of all the comparisons showed no correlation between stomatal resistance and leaf water potential (adj. R^2 = -0.00369). This was also true for both varieties individually. stomatal resistance was linearly related to days since the last irrigation (p = 0.0495) but leaf water potential was not (p = 0.5237).

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Influence of Malolactic Fermentation on Wine Sensory and Chemical Characteristics in Cabernet Sauvignon Wines

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The effects of malolactic fermentation (MLF) on wine aroma profile and chemical properties of Australian Cabernet Sauvignon wine over two vintages were explored by inoculation with up to seven different commercial malolactic starter culture preparations of *Oenococcus oeni*. The effects of initial wine pH on the sensory and chemical changes arising from the MLF treatments were investigated by adjustment of Cabernet Sauvignon wines to pH 3.3 or pH 3.7 prior to MLF induction. The time required for each malolactic culture to complete MLF ranged from 12 days in a lighter style Cabernet Sauvignon wine (13.7% v/v alcohol, initial pH 3.7) to 80 days in the more complex style Cabernet Sauvignon wine (14.8% v/v alcohol, initial pH 3.3). Sensory properties of the wines were investigated by descriptive sensory analysis, using a trained panel. In support of the sensory data, chemical properties of each wine were determined including phenolics and tannin composition, volatile aroma compounds, and organic acids. Significant sensory and compositional differences occurred as a result of the different MLF treatments, including differences in intensity of perceived fruit flavor. Strain dependent changes in the volatile aroma compounds, including the ester profile, were observed. Relative changes in spectral color and tannin properties were dependent upon ML strain and pre-MLF pH.

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