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Technical Abstracts

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Technical Abstracts

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Alphabetical Listing of Presenting Authors

Abstracts here are those submitted and accepted through the 2006 Call for Papers only.

Sessions & Presentations Listing

Wednesday, June 28

Thursday, June 29

Abstracts are in the approximate order as noted in the conference program. Abstracts here are those submitted and accepted through the 2006 Call for Papers only.

Measuring the Tannin-Binding Capacity of Grape Cell Wall Material during Normal and Extended Ripening

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Grape cell wall material from both the skin and pulp has been shown to bind tannin. We have developed a standardized assay to measure the cell wall binding capacity for tannin in order to assess the total amount of tannin that could be removed by cell walls during fermentation. This standardized assay was used to conduct binding capacity measurements of Cabernet Sauvignon fruit from the University of California, Davis experimental vineyard. Vines were sampled during ripening and analyzed for their tannin-binding capacity as well as for skin and pulp cell wall mass. The product of these two parameters gives the total potential capacity for grape cell wall material to remove tannin in the absence of ethanol. Samples used for analysis included normal clusters as well as clusters severed from the vine at 18.5 Brix. Results showed that skin and pulp cell wall mass as well as their tannin-binding capacity remained relatively constant during ripening. This observation relates to the practice of long hang time and suggests that extended ripening probably results in little change in tannin extractability. Skin cell wall mass was nearly twice that of pulp cell wall mass, while skin cell wall binding capacity for tannin was approximately two-thirds that of pulp cell wall. Skin and pulp values for these parameters were combined to calculate a total potential binding capacity of 0.98 mg/berry. This value represents 61% of the average amount of tannin found in Pinot noir fruit.

Effect of Light Exposure on Accumulation of Flavonoids in Pinot noir Fruit and Extraction in a Model System

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Flavonoids in grapes contribute to the color, astringency/mouthfeel, and health properties of wine. The hypothesis being tested was that differences in cluster light exposure would result in variations in the amount and composition of phenolic compounds in the fruit and also in extraction. The trial was established in a vineyard zone of moderate-to-low vine vigor. When berries were ~2 mm in diameter, two clusters on one shoot were enclosed in opaque boxes and two clusters on another shoot were left exposed. Treatment samples (exposed, dark) were collected at veraison and harvest for fruit analysis by HPLC. Model extractions were conducted on 300 g of crushed berries mixed with 300 mL of 40% v/v ethanol for a 48-hr extraction at 37.8°C. No treatment differences were found in berry weight or soluble solids at harvest. Seed flavan-3-ol monomers as well as proanthocyanidin extension, terminal, and total units (nmol/seed) were higher at harvest in dark compared to exposed clusters. Skin proanthocyanidin extension units (nmol/berry) were 77% higher in exposed fruit compared to dark fruit. Proanthocyanidin compositional differences were found in both seed and skin tissues. Cluster exposure also resulted in a large increase in flavonols. Differences in extraction were determined in a model system.

Effect of Postharvest Dehydration on the Phenolic Composition of Pinot noir Grapes and Wine

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In addition to the changes associated with water loss, the postharvest dehydration of grapes also influences flavor development. This study was conducted in order to understand how the phenolics in underripe fruit change during postharvest dehydration. Pinot noir grapes grown in the Willamette Valley of Oregon were harvested at 22 and 24 Brix. Grapes harvested at 22 Brix were divided into three equal lots, with one lot immediately used for wine production and the remaining two lots placed inside an air tunnel with an air speed of 1.0 to 1.8 m s^{-1}, 38% relative humidity, and a temperature of 22°C. The soluble solids content and weight loss were measured daily and wines were made from grapes when they reached 24 and 26 Brix. The soluble solids of grapes increased about 1 Brix per day; therefore, on the third and fourth day the berries reached the desired concentration; weight loss was 14 and 16%, respectively. The results from berry phenolic analysis indicated that per berry anthocyanin amount declined during dehydration. In addition, the composition of tannins isolated from berries changed during this time. Wines made from increasingly dehydrated grapes tended to resemble the composition and flavor profile of wines made from grapes left on the vine (that is, with a longer hang time). The results of this study suggest that positive postharvest flavor development can occur in grapes when harvested early and allowed to dehydrate under controlled conditions before fermentation.

Rapid Prediction of Tannin and Anthocyanin Concentration in Red Wine Fermentations from UV-Visible Spectra

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The critical role of phenolic compounds in wine sensory properties (color and mouthfeel) and therefore perceived quality makes the development of simple, real-time tools to follow their extraction during fermentation an urgent need. At present, the most widely applied and established methods used to quantitate color and tannin in red wine extraction in both winery and research settings rely on colorimetric and chromatographic techniques and can require significant time for sample preparation and analysis. In the past decade, infrared spectroscopy has been examined for the development of predictive methods to measure a variety of grape and wine parameters. To date, researchers have used visible and near infrared spectroscopy with reasonable success to measure pH, sugar, alcohol, organic acids, anthocyanin, tannin, and polymeric pigments. However, limitations in this technology exist both in instrumentation costs and in the resources needed to prepare and validate adequate predictive models. At present,

predictive methods exploiting information from the UV region of the spectrum have not been reported. In this work, partial least squares regression was applied to the UV-visible spectra of juices, fermentation samples, and finished wines. Analysis of data collected in California and Australia suggests that information in the UV-visible spectrum alone is able to quantify not only anthocyanins but also large polymeric pigments, total phenols, and tannin fractions as they are extracted from skins and seeds. Data demonstrating the potential of UV-visible spectra to predict quality parameters in red wines, musts, and juices will be presented.

Rootstocks with Broad and Durable Nematode Resistance

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Grape rootstocks with broad resistance to nematodes are preparing for release. Seedling populations were first selected for their rooting ability and those that rooted were screened sequentially, and then in combination, against four nematodes: the root-knot nematode (*Meloidogyne incognita* race 3), two strains of root-knot nematode that overcome the resistance of Harmony rootstock (*M. arenaria* strain A and *M. incognita* strain C), and the dagger nematode (*Xiphinema index*). This round of testing resulted in six resistant selections: 8909-05, 9363-16, 9365-43, 9365-85, 9407-14, and 9449-27. These selections were then tested against lesion nematode (*Pratyclenchus vulnus*), citrus nematode (*Tylenchulus semipenetrans*), and ring nematode (*Mesocriconema xenoplax*). We also evaluated ring nematode resistance in the parents of the current rootstock candidates and in some other *Vitis* sources. Only one of the rootstock candidates, 8909-05, resists ring nematode. The durability of resistance was tested at higher temperatures, where resistance to root-knot nematode frequently fails; the six selections maintained resistance. Field testing of these rootstock candidates is underway. Nematode population levels are declining in the rootzones of all rootstock candidates, indicating that reproduction of root-knot nematodes is not occurring. However, population levels of ring nematodes at the field site are high on most of the selections, underscoring the need for obtaining new sources of resistance to that nematode. Certification of these rootstocks is expected by spring 2007.

Gene Expression Differences between *Vitis vinifera* and the Disease-Resistant American Grapevine *Vitis aestivalis*

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Microarray analysis provides a global view of the functioning of a plant's genome. Comparing transcription profiles in different grapevines enables us to associate certain phenotypes with specific gene expression patterns, which, in turn, allows us to set up hypotheses about the function of grapevine genes. Using the Affymetrix Vitis GeneChip microarray platform, gene expression profiles were generated in young leaves of *Vitis vinifera* Cabernet Sauvignon and *V. aestivalis* Norton under normal physiological conditions. Statistical exploration of the data indicated that the Vitis GeneChip reliably measured transcript abundance in both grapevines. We identified 9,621 genes whose mRNA was consistently detectable in all six replicates of both grapevines. We found 39 genes that were consistently expressed in *V. vinifera*, but undetectable in *V. aestivalis*, and 183 genes that were consistently present in *V. aestivalis*, but undetectable in *V. vinifera*. A group of genes that were expressed differently between the two grapevine species was also identified. Interestingly, several of the annotatable genes (E value<$1e^{-10}$) whose

transcripts could be specifically detected in *V. aestivalis* played a putative role in defense against pathogens. These results are contrasted with those from a comparative transcriptome analysis study in powdery mildew-challenged *V. vinifera* Cabernet Sauvignon and *V. aestivalis* Norton.

Resistant Grapevine Rootstocks for Control of Armillaria Root Disease

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Armillaria root disease threatens the long-term productivity of California vineyards infested with the pathogen, *Armillaria mellea*, which also infects the roots of forest trees. Where forests are converted to vineyards, *Armillaria* persists in infected roots buried underground. Armillaria root disease causes significantly decreased yield and growth, lower root absorption of macronutrients, delayed ripening of fruit, and eventual death. Soil fumigation is, demonstrably, not the best means of control. An alternative is needed. We evaluated the resistance of eight rootstocks (Freedom, O39-16, Ramsey, Riparia Gloire, St. George, 5C, 110R, 3309C) to infection by *Armillaria*. Green cuttings were rooted in a mist chamber. After a year of growth in the greenhouse, between 15 and 26 rootings per rootstock were inoculated by securing segments of wood colonized by *Armillaria* to their root collars. After three years, they were examined for infection. Freedom had the fewest infected rootings (7%) compared with the other rootstocks ($p < 0.0001$). Rootstocks with the most infected rootings were O39-16, 5C, Riparia Gloire, and 3309C, with 63%, 73%, 79%, and 85%, respectively. St. George, Ramsey, and 110R had intermediate frequencies of infection. Our findings demonstrate that Freedom and, to a lesser extent, St. George, Ramsey, and 110R are relatively tolerant of *Armillaria*. Resistant rootstocks may be a useful component of an integrated management strategy for control of Armillaria root disease. A combination of resistant rootstocks and cultural and biological practices, as opposed to soil fumigation alone, is more likely to maximize the productivity of an infested vineyard over time.

Grape Phylloxera Genetic and Phenotypic Variability as Related to Rootstock Nodosity Feeding

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Grape phylloxera nodosities exist in large numbers (up to 20 nodosities per shovelful of roots) on a few plantings of 101-14 Mgt, Teleki-5C, and 1103P. We bioassayed three 101-14 Mgt-derived phylloxera populations against 26 rootstocks to determine 21-day (one generation) nodosity populations compared with biotypes A and B. Hosts of origin showed greater than 5-fold increase in populations compared with many nonhost of origin assays in which survival overlapped 0.0 population increase. Type A and B tended to do poorly on root types with no *Vitis vinifera* parentage, whereas the 101-14 Mgt phylloxera tended to do well. The reverse was true for root types containing no *V. vinifera* parentage. Single

sequence repeat analysis of DNA from field-collected populations suggest that the host of origin has a strong selection pressure on insect genotypes. These data suggest, first, that 101-14 Mgt phylloxera are genetically distinct from types A and B. Second, abilities on field vines are genetically based, although results are likely influenced by site conditions. Third, *V. vinifera* has nonhost (resistance) characteristics for specific phylloxera strains just as the American *Vitis* species have susceptibility characteristics for specific phylloxera strains. Last, the 101-14 Mgt phylloxera colonies from different locations performed differently in the bioassays, suggesting that the susceptibility and resistance characteristics are complex and not simple phenomena. We plan to extend this work to determine whether seasonal or nodosity/tuberosity strains compound the complexity of phylloxera diversity.

Inception and Progression of Berry Shrivel and Bunch Stem Necrosis in the Napa and Sonoma Valleys

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Berry shrivel and bunch stem necrosis are two disorders that afflict vines in the Napa/Sonoma viticultural areas, and symptomatic fruit is usually dropped in the vineyard before harvest. Both disorders involve the visible shriveling of the berries during ripening. Clusters displaying berry shrivel have a green, healthy-looking rachis. The rachis becomes necrotic in bunch stem necrosis clusters before or at the same time as visible symptoms appear. In the 2005 season, the timing of the disorders was different: bunch stem necrosis clusters became visibly symptomatic throughout the ripening period, from soon after veraison all the way to harvest. Berry shrivel clusters appeared in the vineyard only from September 13 to 17. Compositional analyses of the fruit indicate that, while visible symptoms did not appear in berry shrivel fruit until September, the fruit was markedly different in its concentration of sugars, malic acid, and phenolics from nonsymptomatic fruit shortly after veraison. Berry shrivel is present and affects the development of fruit before visible symptoms become apparent. Compositional data suggests that berry shrivel is not a phenomenon of individual clusters as had been previously thought, but of an entire vine. Healthy-appearing clusters from vines that have berry shrivel clusters, or have a history of berry shrivel in previous seasons, are compositionally different than control clusters from vines with no history of berry shrivel.

Physical and Chemical Properties of Grape Berries as Influenced by Extending Ripening

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The consequences of extending ripening or hang time on fruit composition and physical characteristics are generally understood only in a broad sense; therefore, a study was conducted to examine the effects of extended ripening on the physical and chemical properties of Merlot grape. Grapes were harvested at three different times: (1) at traditional ripeness, (2) 21 days after the first harvest, and (3) 36 days after the first harvest. Grapes were analyzed for changes in physical and chemical properties. Prolonged ripening reduced berry weight but increased the concentrations of soluble solids (Brix). Malic acid concentration declined in the second harvest but increased to levels equivalent to the first harvest. The tartaric acid concentrations of the last two harvests were similar but lower than the first harvest. The titratable acidity of the grapes from the first and third harvests was similar but lower than the second harvest. The pH increased during the extended ripening period but did not differ between the last two harvests. Skin tannins and anthocyanins per gram of fruit increased as did the tannins and phenolics in the seeds in response to extended ripening. The study indicated that the physical and chemical properties of grape berries continued to change during the extended ripening period; such changes may or may not be desirable for making certain styles of wine.

Microbial Utilization of Nitrogen from Vineyard Cover Crops

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Arbuscular mycorrhizal (AM) fungi are one of few groups of soil microbes that have been shown to benefit grapevine nutrition. We have previously shown that external hyphae of AM fungi can serve as bridges for nitrogen (N) transfer from no-till cover crops to grapevines. The objectives of this study were to evaluate the contribution of AM fungi to N uptake from a tilled cover crop, bur medic (*Medicago polymorpha*), and to characterize the soil microbial response to the decomposing cover crop under the influence of mycorrhizal roots or external hyphae. Grapevines were grown in specially designed containers, within which a mesh core was inserted. The mesh allowed mycorrhizal roots (mycorrhizosphere treatment), external hyphae (hyphosphere treatment), or neither to access ^{15}N-labeled cover-crop litter placed inside the cores after 4 months of grapevine growth. Grapevines and soils were harvested 0, 7, 14, and 28 days after addition of the litter. External hyphae increased ^{15}N uptake from the litter (62 μg), but their contribution relative to that of the mycorrhizal roots (927 μg) was minor. We detected similar peaks in soil microbial biomass in the hyphosphere (37 nm g^{-1} soil) and mycorrhizosphere treatments (38 nm g^{-1} soil) after addition of the litter, despite significantly lower microbial biomass in the hyphosphere treatment initially

(10 nm g^{-1} soil). Results suggest that although grapevine roots play a dominant role in the direct uptake of nutrients from tilled cover crops, AM fungi may have a more important role in maintaining soil microbial communities associated with nutrient cycling.

Evaluation of Rootstock Mothervines in Matted and Trellised Growing Systems

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Rootstock mothervines grown for propagation purposes have traditionally been grown in a matted fashion. Some nurseries have adopted the practice of trellising rootstock mothervines, believing that this system produces longer, straighter canes. Furthermore, trellised rootstock vines are said to have greater exposure to sunlight and produce cuttings with greater rooting potential. Trellised systems, however, require significantly more labor to establish and manage than matted systems, which require no training and suppress weeds with their sprawling growth. To evaluate which system produced a greater amount of high-quality cuttings, we evaluated three different genotypes of rootstock mothervines, 420A Mgt (*V. berlandieri* x *V. riparia*), 101-14 Mgt (*V. riparia* x *V. rupestris*), and 110R (*V. berlandieri x V. rupestris*), grown in matted and trellised systems with high and low nitrogen treatments. We then correlated that data to canopy light transmittance and rooting capacity of cuttings. The data collected thus far shows that matted vines produce higher total pruning weights as well as a higher percentage of usable cuttings. The matted systems also required far less labor to manage. However, canopy light transmittance data suggests that canes growing in the trellised systems have lower respiratory loads. Root and shoot production of bench-grafted and callused cuttings indicate that cuttings grown in the more sunlit trellised environments produced larger, heavier roots suggesting that the trellised systems are able to produce cuttings with a greater propensity to root than the matted systems.

Effects of Irrigation Practices and Rootstock on Glycosyl-Glucose in Pinot noir Winegrapes

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Over a two-season period in the Santa Lucia highlands near Soledad, CA, established vines were selected and treated uniformly to investigate the effects of rootstock and irrigation practices on the level of glycosyl-glucose (GG) present in grapes at harvest. The study was done in a commercial vineyard where three contiguous blocks consisted of the same clone (Pinot noir 113) on three rootstocks (101-14, 110R, and 5C). Vines were irrigated weekly from fruit set through harvest at percentages of estimated evapotranspiration during the 2001 and 2002 growing seasons. Irrigation treatments were 100% and 50% evapotranspiration. Clusters were sampled and analyzed for total glycosyl-glucose (TGG) and potential

free glycosyl-glucose (PFGG). Major findings were that rootstock and irrigation practices had a significant influence on PFGG in both years of the study. In 2001, 5C produced a higher level of PFGG than either 101-14 or 110R, and the irrigation treatment of 50% evapotranspiration produced a higher level of PFGG than the control. In 2002, 101-14 and the control irrigation treatment of 100% evapotranspiration produced higher PFGG levels. Also in 2002, rootstock had a significant influence on TGG and the interaction of rootstock and irrigation practices also was significant. Among rootstocks in 2002, 101-14 produced the highest level of TGG, and 101-14 subjected to the control irrigation practice produced the highest level of TGG.

Effect of Hang Time on Yield, Fruit Composition, and Vine Health under Regulated Deficit Irrigation in Syrah Grapevines

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In seeking fully developed flavor for quality wines, hang time has been practiced and harvest delayed in California and other regions in recent years. An experiment was conducted to investigate the effect of hang time on yield, fruit maturation, and vine health under preveraison regulated deficit irrigation (PreV-RDI) in a Syrah vineyard in the San Joaquin Valley, CA. The experiment was conducted as a split-plot factorial of three hang times (at 24 Brix, 28 Brix or when fruit quality started to decline before Brix reached 28, and no harvest) and three PreV-RDI treatments (mid-day leaf water potential of -1.1, -1.3, and -1.5 MPa). Longer hang time resulted in lower yield and more dehydrated berries with higher fruit Brix, pH, glucose and fructose, amino-N, and K but lower TA and malic acid. Biomass degradation also contributed to the decrease in berry fresh weight and yield during longer hang time in addition to dehydration. PreV-RDI was effective in reducing fruit size and enhancing fruit maturation, similar to hanging fruit on the vine longer under conventional irrigation. PreV-RDI and hang time did not significantly affect pruning weight, leaf photosynthesis, and leaf water relations. PreV-RDI increased K content slightly while hang time did not affect the content of any mineral nutrient in dormant canes. It appears that hang time and PreV-RDI had greater impact on yield and fruit composition than on vine vigor and health.

Effect of Abscisic Acid Application on Berry Color Development in Cabernet Sauvignon, Syrah, and Merlot Grapevines

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Abscisic acid (ABA) applied to clusters was reported to increase fruit color by several-fold on some table grape varieties. It was hypothesized that ABA application would also enhance color development in winegrape cultivars to improve fruit quality under adverse climatic conditions. Berries of Cabernet Sauvignon, Syrah, and Merlot grapevines were detached from rachis and submerged for 10 min in ABA solution at 0, 150, 300, 600, and 1200 mg/L at veraison as well as ~3 or

5 weeks postveraison. Incubated berries were then placed at room temperature for 4 days before the skin was analyzed for total anthocyanin. ABA incubation at 600 mg/L induced greatest skin color development in Cabernet Sauvignon and Merlot at veraison but in Cabernet Sauvignon only 3 weeks postveraison. Skin anthocyanin content was correlated linearly to ABA concentration in Syrah at veraison and 3 weeks postveraison, while it peaked at 150 mg/L 5 weeks postveraison. Skin anthocyanin content was not affected by ABA incubation in Cabernet Sauvignon 5 weeks or in Merlot 3 and 5 weeks postveraison. In addition to incubation, clusters of mature Cabernet Sauvignon grapevines were also sprayed with ABA at the same concentrations at veraison in a completely randomized block experiment. ABA sprayed directly onto Cabernet Sauvignon clusters increased skin anthocyanin content up to 40%, which however was much lower than that previously reported for table grape cultivars. It appears that skin color development was more sensitive to ABA application at the earlier date.

Impact of Hang Time on Yield, Fruit Composition, and Vine Health under Different Pruning Regimes and Crop Levels in Syrah Grapevines

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Hang time has been practiced in recent years without much scientific research. An experiment was conducted to investigate the fruit maturation and vine health when fruit was harvested beyond the commonly accepted standard of 22 to 25 Brix under different pruning regimes and crop levels in a Syrah vineyard in the San Joaquin Valley of California. The experiment was designed as a split-plot factorial of three hang times (at 24 Brix, 28 Brix or when fruit quality started to decline before Brix reached 28, and no harvest), two pruning regimes (hand and machine), and two crop levels (with or without 50% cluster thinning at pea-sized berry stage). Berry fresh weight, fruit biomass (dry weight), and yield peaked at 16 to 17 Brix regardless of crop levels. Fruit Brix, pH, glucose and fructose, amino-N, and K increased while TA and malic acid decreased with longer hang time. Lower crop levels due to hand pruning and cluster thinning were associated with lower yield of larger berries with higher fruit Brix, pH, glucose and fructose, malic acid, amino-N, and K. Hand pruning and lower crop level resulted in greater pruning weight. Vines of low crop load were associated with higher N, K, and Mn content while hand-pruned vines had higher Cu, S, and Zn content in dormant canes. Hang time did not affect pruning weight or any cane mineral content. Fruit TA, leaf photosynthesis, and leaf-water relations of mature leaves were not affected by any treatments.

Altering Grapevine Fanleaf Virus RNA to Better Understand Fanleaf Infection, Resistance, and Tolerance

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Vitis vinifera is susceptible to a wide range of pests and diseases including grapevine fanleaf virus (GFLV) and its nematode vector, *Xiphinema index*. This virus/nematode complex causes fanleaf degeneration, a serious grape disease resulting in poor berry set and severe crop loss. *Muscadinia rotundifolia*-based rootstocks (VR hybrids) resist *X. index* feeding, but they allow transmission of GFLV to the susceptible scion. Unexpectedly, the VR hybrid rootstocks possess the unique ability to induce tolerance against fanleaf degeneration in infected scions, despite apparent unimpeded replication of known infectious virus particles. To dissect the molecular mechanisms leading to this induced tolerance, we obtained partial cDNAs of GFLV's two single-strand positive sense RNAs, RNA1 and RNA2, from G. Bruening (Department of Plant Pathology, UC Davis). The full-length clones were constructed and further introduced into an *Agrobacterium* binary vector. We expect the inoculation of plants with an *Agrobacterium* suspension containing a mixture of the RNA1- and RNA2-based plasmids will result in the development of disease symptoms. To confirm the utility of this approach, a green fluorescent protein construct based on RNA2 is being created. This fluorescent tag will allow for tracking and monitoring of GFLV movement from rootstocks through graft unions and into the scion. Alteration of GFLV RNA will also allow us to characterize the infection process, to develop new ways to resist fanleaf with transformed rootstocks, and to understand the ability of VR hybrid rootstocks to induce fanleaf tolerance.

Implementation of Calibrated Near-Infrared Spectroscopy in Precision Viticulture and Selective Harvesting of Winegrapes

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The implementation of precision farming techniques to viticulture is dependent on both the ability to measure physiological benchmarks across a vineyard and an understanding of their spatial relationships to each other. To achieve these ends, a portable near-infrared (NIR) spectrometer was used in conjunction with a GPS/GIS utility to rapidly measure (9 sec per measurement) the differential distributions of Brix, pH, TA, and anthocyanins across several vineyards. In 2003 and in 2004, these parameters were calibrated against standard reference methods using partial least squares analysis. The correlation coefficients for these calibrations were determined to be 0.92, 0.75, 0.76, and 0.82, respectively. In 2005, this system was used to create real-time maturity maps of two commercial Merlot vineyards. Geostatistical procedures were applied and the spatial variability of the aforementioned parameters were examined by variogram analysis. Results showed that anthocyanins had weak (W) to moderate (M) spatial dependence (Cambardella index = 79.35 [W], 60.75 [M]) in both vineyards, allowing us to

predict (map) the anthocyanin content across targeted areas between sampling locations. We then selectively harvested the vineyards according to their predicted anthocyanin levels. Two three-harvest tiers were designated: high, low, and a medium according to the relative distribution of predicted anthocyanins. Colorimetric analysis (hue/intensity), analysis of total anthocyanins (mg/L), and analysis of total phenolics (GAE mg/L) were performed on the wines made from the different tiers. All were consistent with our predicted low to high anthocyanin designations, confirming that our vineyard predictions were accurate and commercially applicable.

Soil Potassium Availability and Management in Relation to Major Soil Types in Napa and Sonoma

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Potassium is an essential nutrient for vine growth and yield. During ripening, potassium (K) accumulates in grape berries. When in excess, K decreases free tartaric acid, increasing the pH of grape juice, must, and wine, resulting in lower wine quality. When K supply is deficient, berry sugar content decreases. Soil potassium supply must meet plant demands and the soil available nutrients must be in the optimal range. However, K availability to the vines varies depending on a range of factors. Ranking high among those factors are soil type, soil management practices, and rootstock affinity for K uptake. Little published information is available on K nutrition in California winegrapes. To date, most research has been conducted in the Central Valley region of California, primarily on table grapes such as the Thompson Seedless variety. Because of the broad range of soil types and microclimates present in the Napa and Sonoma AVAs, there is a need to determine how soil variability influences nutrient availability and ultimately winegrape yield and quality. There is a need for local K fertilization guidelines, based on a better understanding of local soil characteristics, and particularly their textural composition, cation exchange capacity, and mineralogy.

Cluster Abortion in Early Stages of Grapevine Development in Sonora, Mexico

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The yield of a vineyard depends on the number of clusters that are harvested. Some of the potential clusters are lost soon after budbreak when the shoots are ~30 cm. At this stage the clusters do not grow more than 1.5 cm, turn to green yellow, and then fall off. Cluster abscision may be critical in some years. The objective of this work was to compare the cluster abortion in contrasting years in different plants of the cv. Flame Seedless. It was found that cluster abortion was 75% in 2004 and 2% in 2005. In December 2003, after budbreak induction, there were minimum temperatures below 0°C, while in December 2004 there were not. In 2005 there were grapevines in the vineyard that lost their clusters. These

grapevines showed a lower shoot growth rate and smaller leaf size. The plants that aborted their clusters had fewer roots than grapevines that kept their clusters. The nutritional status of both groups of grapevines was similar. Therefore, cluster abortion is promoted by freezing temperatures after the induction of budbreak but also is determined by the plant. The inflorescence primordia may be highly sensitive to the freezing temperatures after the induction of budbreak and under these temperatures they abort. Under good weather conditions the grapevines with poor reserves may allocate their energy to shoot growth rather than to cluster growth.

Freezing Temperatures Inhibit the Action of Hydrogen Cyanamide on Grapevines

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One of the objectives in the viticulture of Sonora, México is to harvest in early May to obtain higher prices. To achieve this goal the grapevines are pruned in December and then hydrogen cyanamide is applied to stimulate budbreak. Hydrogen cyanamide was unable to stimulate uniform budbreak in 2004 apparently because of the freezing temperatures after the applications. The objective of this work was to determine the effect of low temperatures on the action of hydrogen cyanamide. The experiments were conducted in 2004, 2005, and 2006 by exposing cuttings of the cv. Perlette with four buds to low temperatures before or after the application of hydrogen cyanamide from 1.5 to 5%. It was found that budbreak was not stimulated by hydrogen cyanamide 1.5% when temperatures of -9.9°C were interfering one or five days before or after the applications. Hydrogen cyanamide at 3%, but not at 1.5%, stimulated budbreak when the cuttings were exposed to -9.9°C for 6 hours before the application. Hydrogen cyanamide stimulated budbreak at the rate of 1.5% when the time of exposure to -9.9°C was reduced to 4 or 2 hours or when the temperature at which the cuttings were exposed was 1.2°C for 6 hours.

Investigations of Necrotic Unions in Different Clones of Pinot noir on 110R

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Objectives of the research are to demonstrate the biotic nature and molecularly characterize the putative causal viral agent. A small number of vineyards in Napa and Sonoma counties planted largely with noncertified sources of Pinot noir on 110R rootstock contained unthrifty plants. Affected grapevines had red to fall colored leaves and produced short cane growth with little to no fruit. Examination of trunk stems revealed a solid line of necrotic tissues at scion-rootstock junctions, thereby girdling the vines. Molecular assays for the common grape viruses were mostly negative except for the occasional positive for *Rupestris* stem pitting associated virus. Limited surveys in blocks of Pinot noir 667, 2A, and 777 showed

red leaf incidences ranging from 5 to 45%. Two or more trunk samples per block were sacrificed and all had necrotic unions. In October 2004 and again in 2005, an 8-row x 80 plant subblock within a Pinot noir 2A/110R vineyard (at another location; planted 1997) was surveyed, mapped, and disease incidence rose from 2% (14 diseased) to 8% (52 diseased). The rise in disease incidence is suggestive of spread. During August 2005, several collections were graft-inoculated onto healthy test plants of Chardonnay propagated on 10 rootstocks, including 110R. Dormant canes are being processed to purify, clone, and sequence viral dsRNAs.

Effects of High Temperatures on Anthocyanin Composition and Anthocyanin Biosynthetic Gene Expression in Red Winegrapes

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High temperatures inhibit anthocyanin accumulation in grape berry skin. In order to determine the mechanism of inhibition of anthocyanin accumulation due to high temperature, we examined the effects of high temperatures on anthocyanin composition and the responses in terms of gene transcript levels using potted grapevines of *Vitis vinifera* L. cv. Cabernet Sauvignon grown in a phytotron. High-temperature treatment (day temperature, maximum 35°C) was initiated when the berry softening process began. High temperatures reduced the total anthocyanin content to less than half of that in the control berries (grown under 25°C) at 4 weeks after veraison (WAV). HPLC analysis showed that the concentrations of anthocyanins, with the exception of malvidin derivatives (3-glucoside, 3-acetylglucoside, and 3-*p*-coumaroylglucoside), decreased considerably in the berries grown under high temperatures as compared with the control. However, Affymetrix Vitis GeneChip microarray analysis indicated that the anthocyanin biosynthetic genes were not strongly down-regulated at high temperatures. A quantitative real-time PCR analysis confirmed this finding. The mRNA levels of most anthocyanin biosynthetic genes that were exposed to high temperatures increased transiently at 2 WAV and then decreased at 4 WAV; however, the difference in mRNA levels between the high-temperature condition and the control was smaller than the difference in the total anthocyanin content. These data suggest that the decrease in anthocyanin accumulation under high temperatures results from the inhibition of mRNA transcription of the anthocyanin biosynthetic genes and from other factors such as anthocyanin degradation.

Influence of Extended Ripening on Berry Chemistry of Pinot noir Grapes

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This study was conducted to elucidate the changes in berry chemistry as well as the change in berry structure due to extended maturation. Pinot noir Pommard 5 clone on 5C rootstock was studied in a commercial vineyard in Arroyo Grande, CA. Harvests were conducted at 22, 24, 26, and 31 Brix and juice chemistry deter-

mined. Anthocyanins and flavonoids continued to increase throughout the experiment, in contrast to previous reports showing these compounds declining at late maturity. The loss of berry size was largest between 24 and 26 Brix, and the late harvest resulted in a 38% loss of berry weight between the 22 and 31 Brix harvests. Commercial size lots (one ton) were fermented, barreled, and aged in order to simulate industry practices. The finished and bottled wines will be submitted to expert panels for sensory review and to wine consumers in order to array the order of preference from wine industry professionals and consumers.

Using Molecular Markers to Fine-Scale Map a PD-Resistance Gene and Assist Grape Breeding

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In concert with Pierce's disease (PD)-resistance breeding efforts, genetic mapping populations have been created to enable development of genetic markers for selection in the breeding program and to locate the PD-resistance locus prior to characterization efforts. Simple sequence repeat (SSR) markers were used to create genetic maps for these populations with the goal of locating the PD-resistance locus, *PdR1*. The 9621 population was produced from a cross of PD-resistant D8909-15 x PD-resistant F8909-17. The 04-190 population was produced from a cross of *V. vinifera* F2-7 (Cabernet Sauvignon x Carignane) and PD-resistant F8909-08. F8909-08 is a male sibling of F8909-17. In both populations, resistance to PD mapped as a single major locus, segregating 1:1 in the progeny. *PdR1* maps to linkage group 14 on both maps of these full sibling male parents. There are six markers within 10 cM of *PdR1* on the F8909-17 and F8909-08 maps. These markers showed tight linkage to the resistance locus in both populations. A total of six markers were selected (four on one side and two on the other side of resistance locus *PdR1*) to screen progeny from 10 other populations, which derived their resistance from F8909-08. All of these progeny were also tested for PD resistance using our standard greenhouse testing procedure.

Quantification of Erosion Rates for Various Vineyard Management Practices

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Five general management practices were investigated for their potential to erode with high winter rainfall: no till, spring till, spring mow, mulching, and bare rows. Rainfall simulation was used to determine the likelihood for erosion under each of these management strategies. The constituents measured include infiltration rate, sediment runoff concentration, sediment yield, nutrient yield, and time to runoff (the length of time before runoff occurs). Rainfall simulation data coupled with two seasons of rainfall sampling indicates that a no-till strategy results in the least amount of runoff, the lowest sediment yields, and the longest time before erosion occurs. Mulching also resulted in low sediment yields. However, the volume of

runoff was similar to spring mowing. Bare rows yield the highest sediment concentrations, shortest time to runoff, and lowest infiltration rates.

Impact of Timing of Fruit-Zone Leaf Removal on Monoterpenes and Wine Quality of Viognier

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Viognier vines planted in a north-south row orientation were subjected to fruit-zone leaf removal at critical stages of berry development. Leaves were removed by hand from the west side of the vines at bloom, set, and veraison, with a nonleafed control. Grapes were sampled before harvest and analyzed for free volatile terpenes (FVT) and potential volatile terpenes (PVT). Leaf removal had no effect on FVT concentration in berry samples; however, PVT concentration was highest when leafing took place at bloom or set. Leaf removal at veraison resulted in berry PVT concentration that was similar to the nonleafed treatment. Sensory analysis of wines was carried out four months after harvest and no differences in aroma or flavor profile were found across the treatments. That was attributed to the early stage of wine development where fermentation aromas interfered with the sensory attributes of the wines.

Apoplastic Solute Accumulation Accounts for Berry Cell Turgor Loss before Veraison in *Vitis vinifera*

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Veraison has been characterized by softening, sugar accumulation, and a renewal of cell enlargement. Veraison also involves loss of mesocarp cell turgor pressure. We hypothesized that this loss was associated with an accumulation of apoplastic solutes in mesocarp tissue before veraison. Greenhouse-grown Chardonnay and field-grown Cabernet Sauvignon berries were used to test this hypothesis. Apoplastic sap was extracted from the berries using either a centrifuge or a pressure membrane technique, and thereafter the apoplast osmotic potential was measured. The apoplast osmotic potential measured with pressure membrane technique was similar to that measured with the centrifuge technique. In Chardonnay, the apoplast osmotic potential was -0.20 MPa at late stage I and decreased to -3.37 MPa by the middle of stage III. Mesocarp cell turgor pressure was 0.32 MPa at late stage I and reached 0.06 MPa with the decrease of apoplast osmotic potential by the middle of stage III. The osmotic potential difference between the apoplast and the mesocarp cells decreased from late stage I and was maintained around zero after the onset of ripening. Therefore, we conclude that the high concentrations of solutes are accumulated into the apoplast of the mesocarp tissue before veraison and cause the decrease in mesocarp cell turgor pressure. Because these events preceded veraison, our findings suggest that they play an important role in the events of cellular metabolism that lead to veraison.

Influence of Canopy Management Practices and Growing Region on Flavor of Cabernet Sauvignon

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Vine microclimate and cluster location (side of vine row) were evaluated for their effects on composition and flavor of Cabernet Sauvignon wines. Vines with six different cluster exposures and canopy densities were grown in four different regions of California (Lodi, Napa, Paso Robles, and Gonzales). Wines made from each treatment were analyzed chemically and by sensory descriptive analysis. The different canopy treatments produced wines which differed significantly in some vegetal and fruity characters as well as in perception of astringency. Also, significant interaction terms indicated that the various canopy treatments had differential effects on the sensory properties for the different growing regions. For example, higher vegetal and astringency intensity were found in wines made from grapes grown in rows with morning sun exposure in the coolest area (Gonzales). High levels of vegetal character were also found in wines from the warmest region (Lodi). Vegetal characters were correlated with 2-methoxy-3-isobutyl pyrazine (MIBP) concentration in some, but not all, cases. In conclusion, there is a complex interaction among growing region, row orientation, and vine microclimate, which influences sensory and chemical composition in wines. Therefore, any viticultural efforts made to manipulate wine properties should consider the unique conditions that may exist at any site, including climate, soils, and vine microclimates.

Evaluating Changes in the Virulence of Root-knot Nematode on *Vitis riparia* and Rootstock Hosts

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Fluctuations of root-knot nematode (RKN) virulence have made it difficult to map *Vitis champinii*'s single dominant gene for resistance in a *V. champinii* cv. Ramsey x Riparia Gloire population. Variation in RKN virulence may also have detrimental implications for the durability of grape rootstock resistance sources. Riparia Gloire (*V. riparia*) was shown to be susceptible to RKN in the past, but because of a putative loss of virulence, its feeding ability has declined in recent screens. The feeding ability of three RKN populations, *Meloidogyne incognita* strain 3 and Harmony C and *M. arenaria* strain Harmony A, was tested with similar results. New virulent populations were selected in controlled greenhouse experiments by inoculating Riparia Gloire with juvenile RKN and harvesting the small number of resulting egg masses in an attempt to resurrect the virulence of these RKN strains. Three progressive selections were performed and genomic characterization, using AFLP and SSR markers, was carried out at each stage to track the population and look for allele changes in response to host selection. Various accessions of *V. riparia* and other *Vitis* species and rootstocks, representing a range of susceptibility to RKN, were also used in these experiments to

observe changes in feeding patterns of newly selected strains. The potential reasons for loss and then subsequent gain of virulence of RKN and the genomic characterization of virulent and avirulent strains are examined.

Mapping Multiple Rootstock Traits in a Ramsey X and Riparia Gloire Population

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The first linkage map derived from two commercial rootstocks has been created as a tool for mapping multiple rootstock traits. The SSR-based consensus map was based on segregation data from 188 F1 individuals from the '9715' cross Ramsey (*V. champinii*) x Riparia Gloire (*V. riparia*). These two rootstock parents represent extremes in a number of rootstock traits. Ramsey is used in vineyards as a nematode-resistant rootstock that tolerates drought, salt, and lime and induces high vigor in scions, which can lead to poor fruit and wine quality if the canopy cannot be managed. Ramsey's ability to induce high vigor is likely based on its deep plunging root system. Conversely, Riparia Gloire is a low vigor, easily propagated rootstock. It lacks, however, durable resistance to nematodes, drought, and lime. Riparia Gloire's low vigor is likely due to its shallow rooting architecture. In addition, Ramsey is pistillate flowered and Riparia Gloire's flowers are staminate. Because of the diverse nature of its parents, this Ramsey x Riparia Gloire population is expected to segregate for many important rootstock traits. We report progress toward mapping major genes for root-knot nematode resistance and sex expression as well as QTLs for root architecture and ease of propagation.

Abscisic Acid Applications Improve the Color of Crimson Seedless Table Grapes

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The fruits of Crimson Seedless fail to color adequately in many California table grape vineyards. To some extent, poor coloration may be overcome by applying ethephon, but the effects on color are inconsistent, and ethephon often induces softening, a negative side effect. It has been shown that abscisic acid (ABA) can increase the skin anthocyanin concentration of some grape cultivars, but its high cost has prevented further investigation. Recently, a new ABA formulation method was developed that may allow for commercial application: therefore we evaluated different application concentrations and timings, over three seasons, on Crimson Seedless, one of the more important red table grapes in California. In 2003 ABA concentrations of 1,000 mg/L applied at veraison markedly increased the skin anthocyanin concentration; in fact the grapes were excessively colored. In 2004, lower concentrations were used and the compound was applied at different times. Uncommonly cool weather in 2004 lead to general good coloration of grapes, yet we were still able to observe increases in the anthocyanin content of

grapes treated with 150 or 300 mg/L ABA at veraison or postveraison. These treatments were repeated in 2005 and increases were again observed in anthocyanin content. Using a chroma meter, it was found that skin anthocyanin concentrations had a highly significant, curvilinear relationship with hue angle and lightness of the berries. In general, few effects were observed on soluble solids, acidity, or pH. Firmness of the berries was affected by ABA similar to ethephon treatments, but anthocyanins and color showed a superior response to ABA.

Effect of Rootstocks on Anthocyanins and Tannins in Grapes and Wine

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Rootstocks have been used to overcome phylloxera, nematodes, site environmental constraints, and control plant size. Little is known about their effect on vine physiology and there are no reports on their impact on fruit and wine phenolic composition. The purpose of this study was to examine the role that rootstocks have in determining fruit and wine chemistry. A rootstock trial was established at Oregon State University's research vineyard in 1997. Pinot noir was grafted onto 19 different rootstocks and compared to ungrafted vines in a completely randomized block design. Pinot noir wines were made from six selected rootstocks in 2004 and from all 20 different scion-rootstock combinations in 2005. Skin tannin production was independent of the vigor imposed by the different rootstocks. During three consecutive seasons, skin tannin was increased for 420A, 110R, and 1616C, while 3309C, Riparia Gloire (RP), and ungrafted vines had the lowest amounts. Rootstocks 420A, SO4, and 5C had higher levels of anthocyanins. Variations in the molecular weight of the tannin were also present. No significant relationship between berry weight and the level of skin tannin or total anthocyanin was found. In 2004, wines made from vines grafted to 110R, 3309C, and RG were preferred by tasters, although 3309C and RG wines had higher hue and tended to have lower color density. Results suggest that changes in fruit composition are not merely the result of the crop size and vigor conferred by the rootstock but that there is actually a direct effect of the rootstock on fruit and wine chemistry.

Progress toward Understanding Induced Tolerance to Fanleaf from *Muscadinia rotundifolia*-based Rootstocks

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Grapevine fanleaf virus (GFLV) is vectored from root-to-root by the dagger nematode, *Xiphinema index*. The combined damage from GFLV infection and by *X. index* feeding results in fanleaf degeneration. Disease symptoms of this complex include foliar deformation and chlorotic patterning, fasciation of shoots, zigzagging internodes, reduced vigor, and most importantly to growers, severe reduction in seed and fruit set, accompanied by irregular fruit development. Long-term field trials have demonstrated that the *Vitis vinifera* x *Muscadinia rotundifolia*

(VR) hybrid rootstock, O39-16, induces tolerance to fanleaf degeneration in their fruiting scions. While these rootstocks have excellent *X. index* resistance, nematode probing for feeding sites results in GFLV movement into the roots, where virions move into the vascular system and up into the scion. We have proven that these virions are infectious, yet disease symptoms are suppressed. Experiments using nuclear magnetic resonance imaging and HPLC are underway to test the hypothesis that root-based cytokinins are responsible for induced tolerance to GFLV. Additionally, a genetic linkage map based upon segregation of simple sequence repeat (SSR) molecular markers is being created from a population of about 100 F1 *V. vinifera* x *M. rotundifolia* progeny. This will be the first *V. vinifera* x *M. rotundifolia* genetic map published, and it will be useful for identifying quantitative trait loci (QTL) associated with induced tolerance as well as many other economically important traits derived from *M. rotundifolia*.

Influence of Rootstock on Must Nitrogen and Sensory Quality of Merlot

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Fruit grown on vigorous rootstocks can have higher amino acid (AA) contents than fruit grown on intermediate and low vigor rootstocks, but not consistently across varieties. In addition, sensory analyses have shown clear differences in flavors and aromas between vintages with different AA profiles. Nonetheless, there is no clear understanding of the role rootstocks play in must quality, or the specific relationship between AA profiles and sensory characteristics of the resulting wines. The purpose of this study was to examine must nitrogen status and the resulting sensory attributes of Merlot grafted onto two popular rootstocks that differ in vigor, 1103P and 101-14 Mgt. The experiments were conducted at Oakville in Napa Valley, California. Amino acid analyses showed that Merlot growing on 101-14 Mgt rootstock had significantly less amino nitrogen than Merlot grown on 1103P. The concentrations of yeast assimilable nitrogen compounds (YANC) were 55% less on 101-14 Mgt, and fermentations achieved dryness one week later than 1103P. Preliminary descriptive sensory analyses showed differences in aromas of Merlot grown on these two rootstocks. Our results suggest that 1103P can absorb and translocate more nitrogen compounds to the scion than 101-14 Mgt and that this may help to explain the different sensory attributes of wines made from grapes grown on these two rootstocks.

Early Assessment of Geotropic Root Angle in Grape Rootstocks as a Method to Predict Vigor Induction in the Field

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Data on the geotropic root angle of 120 progeny from a cross of Ramsey x Riparia Gloire were produced and used to establish a method of early selection for rooting angle in breeding of grape rootstock. Rooting angle is thought to play a key role in the level of vigor that rootstocks induce in scions, with deeper root sys-

tems inducing more vigor. We compared rooting angles from herbaceous cuttings to those produced on dormant rootings. The Ramsey and Riparia Gloire parents performed consistently both as green cuttings rooted in perlite and as mature dormant cuttings rooted in the field for six months. Ramsey produced deep average root angles on herbaceous (59.6 degrees) and on dormant (54.4 degrees) cuttings. Riparia Gloire produced shallow average root angles on herbaceous (27.4 degrees) and on dormant (23.2 degrees) cuttings. Data relative to the 120 seedling progeny are currently being evaluated for correlation of results between green and dormant cuttings, for distribution pattern, and for segregation pattern. Preliminary results show that some of the genotypes differ significantly in root angle from one or the other parent.

Occurrence and Importance of *Botryosphaeria* spp., a Fungal Pathogen Associated with Grapevine Cankers in California

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Botryosphaeria species have recently been identified as important grapevine pathogens worldwide. *Botryosphaeria* spp. infect grapevines through fresh pruning wounds and have frequently been isolated from vines showing decline or dieback symptoms. To date, grapevine cankers in California had been thought to be associated mainly with *Eutypa lata* and *Botryosphaeria rhodina*, causal agents of *Eutypa* dieback and Bot canker, respectively. To assess the occurrence, importance, and distribution of *Botryosphaeria* spp., 166 vineyards of different ages among the main table-raisin and winegrape cultivars from 21 different counties were surveyed throughout the state. Fungal isolations were made from 1,735 samples showing typical perennial cankers collected from spurs, arms, cordons, and trunks. *Botryosphaeria* spp. were found in all counties surveyed and they were the most common fungal pathogens isolated from grapevine cankers in North Coast, Central Coast, South Coast, mountain counties, northern San Joaquin Valley and Southern California (Riverside County) grapevine production areas. *Eutypa lata* was more prevalent than *Botryosphaeria* in the four counties of Napa, Solano, San Joaquin, and Stanislaus. *Phomopsis viticola* was the most predominant fungus associated with cankers in the southern San Joaquin Valley. Morphological identification along with DNA analysis of the ITS and beta-tubulin gene regions showed that at least seven *Botryosphaeria* species occur in grapevines in California, including *B. rhodina*, *B. obtusa*, *B. stevensii*, *B. australis*, *B. parva*, *B. lutea*, and *B. dothidea*. These findings indicate the role of *Botryosphaeria* species in grapevine health needs to be more carefully considered.

Interactions among Weeds, Ants, and Obscure Mealybug in Central Coast Vineyards

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Obscure Mealybug (OMB), *Pseudococcus viburni* (Signoret), is a major insect pest of grapes on the Central Coast. It makes use of weeds as alternate hosts during the growing season as well as in the winter. Mealybug infestations are exacerbated by the presence of ants, which protect the mealybug from natural enemies and distribute mealybug among host plants. In 2004 we undertook a two-year field study to observe the association of OMB with the Argentine ant, *Linepithema humile,* and the effect of eliminating alternate weed hosts for OMB on grape infestation in Central Coast vineyards. The experimental design was a split plot where the presence or absence of weeds was the main plot factor and the presence or absence of ants was the subplot factor. Ant density was measured weekly. The presence or absence of OMB on common spring and summer weed roots was recorded, and grape cluster infestation by OMB at harvest was analyzed. A greenhouse study confirmed the broad host range of OMB on a variety of weed and cover crop species. Most vineyard weed species were found to be hosts for OMB. Results indicate that weed control had no impact on OMB infestation of grape clusters but that ant exclusion played a significant role. We conclude that weed management is not a viable cultural control for OMB.

Irrigation Management during Extended Maturation in Winegrapes: Effects on Yield, Fruit and Wine Composition, and Vine Physiology

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The effect of irrigation regime during extended maturation on yield components, berry and wine composition, and vine physiology was studied in deficit-irrigated, field-grown Cabernet Sauvignon (Sonoma County) and Merlot (Madera County) grapevines in California. Three drip-irrigation treatments were implemented when the fruit reached 20 Brix: 0.5, 1.0, and 1.5 ETo in Sonoma and 0.7, 1.0, and 1.3 ETo in Madera. Fruit was sampled on a weekly basis after reaching 22 Brix, and wines were made every two weeks until commercial harvest. Soil water content, stem and leaf water potential, and photosynthesis were measured diurnally several times during the ripening period. Leaf chlorophyll abundance was measured on three occasions and leaf area at initiation of irrigation treatments and one week after commercial harvest. Irrigation levels had a small but significant effect on vine yield components at both locations. Berry weight, cluster weight, and total vine yield generally increased with increased levels of irrigation. The irrigation treatments had only minor effects on fruit and wine composition. There were no effects on vegetative growth or chlorophyll abundance. These preliminary results indicate that irrigation management during the extended maturation period is a promising tool to mitigate potential yield losses due to berry dehydration.

Occurrence and Importance of *Botryosphaeria* spp., a Fungal Pathogen Associated with Grapevine Cankers in California

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Botryosphaeria species have recently been identified as important grapevine pathogens worldwide. *Botryosphaeria* spp. infect grapevines through fresh pruning wounds and have frequently been isolated from vines showing decline or dieback symptoms. To date, grapevine cankers in California had been thought to be associated mainly with *Eutypa lata* and *Botryosphaeria rhodina*, causal agents of *Eutypa* dieback and Bot canker, respectively. To assess the occurrence, importance, and distribution of *Botryosphaeria* spp., 166 vineyards of different ages among the main table raisin and winegrape cultivars from 21 different counties were surveyed throughout California. Fungal isolations were made from 1,735 samples showing typical perennial cankers collected from spurs, arms, cordons, and trunks. *Botryosphaeria* spp. were found in all counties surveyed and they were the most common fungal pathogens isolated from grapevine cankers in North Coast, Central Coast, South Coast, mountain counties, northern San Joaquin Valley, and Southern California (Riverside County) grapevine production areas. *Eutypa lata* was more prevalent than *Botryosphaeria* in the four counties of Napa, Solano, San Joaquin, and Stanislaus. *Phomopsis viticola* was the most predominant fungus associated with cankers in the southern San Joaquin Valley. Morphological identification along with DNA analysis of the ITS and beta-tubulin gene regions showed that at least seven *Botryosphaeria* species occur in grapevines in California, including *B. rhodina*, *B. obtusa*, *B. stevensii*, *B. australis*, *B. parva*, *B. lutea*, and *B. dothidea*. These findings indicate the role of *Botryosphaeria* species in grapevine health needs to be more carefully considered.

Genomic Analysis of *Oenococcus oeni*

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Oenococcus oeni is a unique lactic acid bacteria isolated solely from fruit-mash and winery environments. Unlike many other lactic acid bacteria, *O. oeni* is tolerant to both high levels of alcohol and low pH conditions, making it ideal for performing malolactic conversion, a key secondary fermentation in the production of wine. In collaboration with Joint Genome Institute, the genome sequence of *O. oeni* PSU-1 has been determined. The complete PSU-1 genome is 1,780,517 nucleotides with a GC content of 38%. 1701 open reading frames could be predicted from the sequence, of which 75% were functionally classified. Consistent with its classification as an obligately heterofermentative lactic acid bacteria, PSU-1 genome encodes all the enzymes for the phosphoketolase pathway. Genes and pathways involved in production of flavor compounds in wine, such as the malolactic conversion, citrate utilization, glycoside activity, and catabolism of amino acids, were readily identified. Array-based comparisons of 10 *O. oeni* isolates obtained from diverse winemaking regions indicate a high level of genome conservation, with all strains possessing at least 96% of the genes present in PSU-1. Strains appeared to cluster into three groups, with no relationship between geographic locale and cluster membership. The lack of diversity apparent in the *O. oeni* species suggests a wide distribution of strains coincident with winemaking practice. The completion of the *O. oeni* genome marks a significant new phase for research on the malolactic fermentation in wine, whereby the physiology, diversity, and performance of *O. oeni* starter cultures can be more rigorously examined.

Functional Analysis of the Malolactic Wine Yeast ML01

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Most red wines and some white wines undergo the bacterial malolactic fermentation that catalyses the bio-conversion of L-malate to L-lactate. The malolactic fermentation usually takes place after the alcoholic fermentation. Malolactic bacteria often find it difficult to grow under adverse conditions that exist after alcoholic fermentation (such as high alcohol, low pH, low temperature, must depleted of nutrients). We have constructed a genetically stable industrial strain of *Saccharomyces cerevisiae* by integrating the *Schizosaccharomyces pombe* malate permease gene (*mae1*) and the *Oenococcus oeni* malolactic gene (*mleA*) under control of the *S. cerevisiae PGK1* promoter and terminator sequences into the *URA3* locus of an industrial wine yeast strain. The malolactic yeast strain, ML01, is fully functional and decarboxylates 5.5 g/L of malate in Chardonnay grape must during the alcoholic fermentation. The malolactic yeast will benefit wineries since it will prevent sluggish and stuck malolactic fermentations and subsequent spoilage of wines. Moreover, the color of red wines produced with the malolactic yeast is much more intense compared to wines produced with bacterial malolactic fermentation. This is particularly useful for the production of Pinot noir wines. The appli-

cation of the ML01 malolactic yeast will also minimize or prevent growth of lactic acid bacteria capable of producing allergens and at least ensure a reduction, or elimination, of these allergens from wine. The ML01 yeast enjoys Generally Regarded As Safe (GRAS) status from the FDA and is the first genetically enhanced yeast that has been commercialized.

New Zealand Sauvignon blanc: Sensory and Chemical Analysis of Unique Characteristics

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"Typicality" is a term used to convey those qualities and flavor characteristics that can be expected from a region. In the wine industry in France, wine is purchased based on the region rather than a type. New Zealand Sauvignon blanc has a unique style different from its French origin in Sancerre and Bordeaux regions, and our goal was to determine what flavors make New Zealand Sauvignon blanc unique. A trained sensory panel identified some key flavors in Sauvignon blanc wines from France, South Africa, Australia, United States, and New Zealand. Sixteen characteristics were identified and measured, including sweet sweaty passion fruit, tropical, capsicum, passion fruit skin/stalk, broom/cat's urine, bourbon, and mineral/flinty. Principal component analysis was used to quantify data based on region/country. Marlborough Sauvignon blanc wines were driven by tropical and sweet sweaty passion fruit characteristics, while French and South African Sauvignon blanc wines were described as having flinty/mineral and bourbonlike flavors. Chemical analyses of these wines also showed that Marlborough wines were greater in the sweet sweaty passion fruit and capsicum reference compounds. This is a preliminary study in a larger project characterising the flavor of New Zealand Sauvignon blanc. Future research will continue to explore regional effects as well as other factors affecting flavor development in New Zealand wines.

Quantitative Response of *Saccharomyces cerevisiae* to Temperature Fermentation

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Temperature is a key control parameter of alcoholic fermentation. Using continous cultures to allow well-defined physiological features, the growth of a lab and a wine *Saccharomyces cerevisiae* strain at 15°C and 30°C were compared. Irrespective of the strain used, we found a significantly higher nitrogen to biomass yields at 15°C, as compared with 30°C, indicating a less efficient nitrogen metabolism at low temperature. The fermentative capacity, measured as specific CO_2 production rate, was 20% slower at the low temperature. Transcriptome and fluxome analyses confirmed these results. Particularly, sugar transporter genes and degradation of alantoin (DAL) genes, responsible for allantoin degradation and purine utilization as nitrogen source, were differentially expressed at both temperatures.

White Wine Aging: The Role of Ascorbic Acid and Bottle Storage Conditions

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A Riesling and a wooded Chardonnay wine were bottled with or without ascorbic acid, sealed with four different closures (screwcap, two different natural corks, and one synthetic) and held either upright or inverted under controlled temperature and humidity conditions for five years. Spectral measures of wine color, the content of sulfur dioxide and ascorbic acid, and sensory analysis of color and aroma were undertaken throughout the storage period. Six months after bottling the addition of ascorbic acid had little effect on wine flavor. After storage for three years or more, wines with ascorbic acid addition were less oxidized and had more fresh fruity aromas than those without. Chardonnay wines with added ascorbic acid were less brown in color and had less overall color intensity compared to wines without ascorbic acid. For Riesling wines, addition of ascorbic acid resulted in greater yellow color after three years. The A420 measurements did not always correlate with the brown scores obtained by visual assessment. Wines sealed with the synthetic closure were relatively more oxidized in aroma, brown in color, and low in sulfur dioxide compared to wines held under the other closures. A reduced aroma was discernible in the wines sealed under the screwcaps. Bottle orientation had little effect on the composition and sensory properties of the wines examined. For both wines, after five years storage, the concentration of sulfur dioxide was statistically significantly higher in wines to which ascorbic acid was added at bottling than those without addition.

Aroma Profile Characterization of Different Strains of *Brettanomyces bruxellensis* in Model Wine

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Dekkera/Brettanomyces bruxellensis metabolism is known to produce the volatile phenols 4-ethylphenol, 4-ethylguaiacol, and 4-ethylcatechol from the hydroxycinnamic acid precursors *p*-coumaric, caffeic, and ferulic acids. These compounds contribute to wine off-flavors described as Band-Aid, burnt plastic, barnyard, and horse sweat. However, some of the odors produced by *B. bruxellensis* are still not chemically characterized. Previous studies have found differences among strains of *B. bruxellensis* both in the production of the volatile ethylphenols and in the consumption of the hydroxycinnamic acid precursors. In this study we characterized the aroma profile of 10 strains of *B. bruxellensis* isolated from wines. The strains were grown in model wine. The model wine media, also used as control, was prepared by fermentation of model juice with a commercial *Saccharomyces cerevisiae* strain. Specific odorants in the model wines were identified by gas chromatography-olfactometry. Each *B. bruxellensis* strain showed a distinct odor profile. A citrus odorant was characteristic of all the strains. Some of the odorants identified in the control wine were modified differently by the different *B. bruxellensis* strains. As expected, odors like Band-Aid and clove characterized all the strains, but smells identified as leather and earthy were characteristic of only some of them. These experiments demonstrate clearly that individual strains of *B. bruxellensis* have different odor profiles. These studies also show that there are additional odorants produced by *B. bruxellensis* that have not previously been chemically identified.

Influence of the Timing of Starter Culture Inoculations on Alcoholic and Malolactic Fermentations in Wine

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Malolactic fermentation (MLF), performed by *Oenococcus oeni*, is important to the flavor of wines, especially in cool-climate winemaking regions, since it can reduce the excessive acidity common to cool-climate grapes. Enologists are interested in determining the best procedure for successful malolactic fermentation that proceeds as quickly as possible and produces attractive qualities in wines, without the production of off-flavors such as acetic acid. Studies have demonstrated strain-dependent negative effects from yeast metabolites in wine and from nutrient competition on MLF bacteria, using defined medium and wines. We studied the influence of the timing of yeast and bacteria starter culture inoculations on alcoholic and malolactic fermentations in a Chardonnay juice. Two strains of yeast were paired with two different bacteria strains. Comparisons of simultaneous yeast and malolactic fermentations and temporally separated fermentations (yeast followed by bacteria) were made. The three inoculation conditions were inoculations of yeast and bacteria performed simultaneously, inoculation of bacteria immediately after completion of primary

fermentation, and bacteria inoculation two weeks after primary fermentation finished. Juice and wine analyses, prior to and following MLF, include pH, titratable acidity, and organic acids (malic, lactic, and acetic acids), sugars, ethanol, primary amino nitrogen, ammonia, and sulfur dioxide concentrations. Growth of the bacteria and yeast and rate of malolactic conversion were also followed.

Effect of Light Exposure on Accumulation of Flavonoids in Pinot noir Fruit and Extraction in a Model System

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Flavonoids in grapes contribute to the color, astringency/mouthfeel, and health properties of wine. The hypothesis being tested was that differences in cluster light exposure would result in variations in the amount and composition of phenolic compounds in the fruit and also in extraction. The trial was established in a vineyard zone of moderate-to-low vine vigor. When berries were ~2 mm in diameter, two clusters on one shoot were enclosed in opaque boxes and two clusters on another shoot were left exposed. Treatment samples (exposed, dark) were collected at veraison and harvest for fruit analysis by HPLC. Model extractions were conducted on 300 g of crushed berries mixed with 300 mL of 40% v/v ethanol for a 48-hr extraction at 37.8°C. No treatment differences were found in berry weight or soluble solids at harvest. Seed flavan-3-ol monomers as well as proanthocyanidin extension, terminal, and total units (nmol/seed) were higher at harvest in dark compared to exposed clusters. Skin proanthocyanidin extension units (nmol/berry) were 77% higher in exposed fruit compared to dark fruit. Proanthocyanidin compositional differences were found in both seed and skin tissues. Cluster exposure also resulted in a large increase in flavonols. Differences in extraction were determined in a model system.

Developments of Aroma and Aroma Precursor in Pinot noir Grapes by Stir Bar Sorptive Extraction–Gas Chromatography-Mass Spectrometry

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Flavor is one of the most important aspects of wine quality, which is not only affected by winemaking but also controlled by grape aroma and aroma precursors. However, the developments of these aroma and aroma precursors in grapes are not fully understood, especially for Pinot noir grapes. In this study, a sensitive and rapid quantification method using stir bar sorptive extraction–gas chromatography-mass spectrometry (SBSE–GC-MS) has been developed to investigate the aroma and aroma precursors in grapes. More than 20 compounds were analyzed, including linalool, geraniol, nerol, β-damasconone, β-inone, vanillin, and ethyl vanillate, which have been reported as important aroma compounds in Pinot noir wine. Calibration curves were built using pure standards and internal standards in an acidic buffer, and the correlation coefficient (>0.95) and RSD (<15%) of the calibration curves were calculated based on six replicate samples. The free

form of aroma compounds was directly analyzed in grape juice, and the aroma precursors were analyzed after enzyme and mild acid hydrolysis. Three years of grapes were investigated by this method, and the aroma and aroma precursors were quantified to show the development during grapegrowing.

Identification of Genes Involved in Suppressed Hydrogen Sulfide Production in *Saccharomyces cerevisiae*

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Hydrogen sulfide (H_2S) production during alcoholic fermentation, by *Saccharomyces cerevisiae,* is among the most prevalent problems negatively affecting wine quality. Past research has demonstrated that although there are numerous environmental and nutritional factors impacting the level of H_2S produced, yeast strain and genetic background are the largest variables. To better understand the mechanisms and pathways through which H_2S is formed, and to develop future prevention or management strategies, a screen of the yeast deletion strain set, comprised of 4,827 mutants, was performed with lead acetate strips to identify genes responsible for suppressed H_2S. This deletion set includes null mutants of all known nonessential genes in the *Saccharomyces* genome. Hydrogen-sulfide-producing null mutants were identified through the color change on a lead acetate strip ranging from light tan to black resulting from the precipitate formed as the H_2S bound to the lead acetate. Thirteen null mutants were identified as producing increased levels of H_2S, compared to the wild type, indicating genes involved in preventing the release of volatile sulfur compounds. Of the 13 genes, five genes were involved in sulfur containing amino acid or precursor biosynthesis and directly associated with the sulfate assimilation pathway. Two genes were associated with additional amino acid and vitamin biosynthesis or transport. The remaining genes were involved in cell membrane integrity, cell energy regulation and balance, and other less direct metabolic functions. Level of hydrogen sulfide production of each of the 13 positive strains is currently being characterized and evaluated in response to various nutritional conditions.

Use of GFP-Tags to Study Hexose Transport Protein Expression in a Wine Strain of *Saccharomyces cerevisiae* during Fermentation

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The transport of sugars across the plasma membrane is the critical step in the utilization of glucose and fructose by yeast cells during wine fermentation. *Saccharomyces cerevisiae* possess a large number of genes homologous to the hexose transporters (*HXT1* to *HXT17*, *GAL2*, *SNF3*, and *RGT2*), yet only Hxt1p through Hxt7p are considered to be the major hexose transporters during fermentation. These seven transporters differ in their substrate specificities and affinities, as well as their expression patterns and regulation, with glucose being the primary factor controlling expression. During wine fermentation, yeast faces

highly changing environmental conditions, as the sugar concentration drops from a high level (≈200 g/L) to less than 2 g/L while the ethanol content increases significantly, and most of the sugars are fermented by nitrogen-starved cells. Therefore, yeast must maintain strict regulation of *HXT* expression over the course of a typical fermentation in order to adapt. In this research we have constructed chromosomal fusions to *HXT1* through *HXT7* using the Green Fluorescent Protein from *Aequoria victoria* to follow protein localization and turnover. Strains expressing these individual fusions were subjected to fermentation of synthetic grape juice containing variable amounts of assimilable nitrogen and/or sugars to observe differences in *HXT* expression in cells under stress. Hxt1p was observed early in the fermentation, while Hxt3p and Hxt5p were the most abundant proteins throughout the fermentation. Hxt6p and Hxt7p were observed late and showed interesting cellular localization patterns.

Effect of Amino Nitrogen on *Brettanomyces/Dekkera* Growth and Aroma Production

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Growth and aroma production by five strains of *Brettanomyces bruxellensis* yeast were evaluated in three different versions of a minimal media with an addition of one of 24 different organic substrates containing nitrogen. The media were differentiated by the presence or absence of a carbon substrate and presence or absence of an inorganic nitrogen substrate. The substrates used were the 20 α-amino acids and commercial fermentation additives Superfood, Fermaid-K, Leucofood, and yeast extract. L-Cysteine inhibited growth in all treatments. Aromas common to all strains were yeasty, sweaty, cheesy, and floral. The strains mainly differed in levels of these aromas produced. The treatments containing sulfur-based α-amino acids produced unique aromas. Floral aromas were particularly strong in the treatments containing L-phenylalanine. Aroma production was also evaluated in 78 treatments of a Cabernet Sauvignon wine with an inoculum of one of the five strains of *Brettanomyces bruxellensis* yeast and addition of one of 12 different α-amino acids. Only one strain exhibited colonies on YM agar plates. All five strains exhibited cell counts, as measured by quantitative PCR, with two having counts exceeding 10^4 cells/mL. By nose, treatments containing these two strains differed by strain, but not by amino acid addition. Aroma compound profiles were also evaluated by solid-phase microextraction and gas chromatography-mass spectrometry.

Assessment of Astringency in Washington State Red Wines Using Sensory and Chemical Methods

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Astringency is one of the most important sensory attributes in red wine and is attributed to the presence of phenolic compounds, specifically tannins, in the wine. The objective of this study was to provide a deeper understanding of the relationship between chemical astringency and consumer perception of astringency. A survey of Washington State red wines was conducted to determine their polymeric pigment levels using chemical methods, form groupings based on these analytical tannin values, and relate these groupings to sensory evaluation. Over two hundred red wines, primarily Cabernet Sauvignon, Merlot, and Syrah, were collected from Washington State wineries. Using a protein precipitation assay with bisulfite bleaching, wines were analyzed for tannin concentration, total polyphenolic concentration, large polymeric pigment content, and small polymeric pigment content. Tannin concentrations were expressed as catechin equivalents (mg/L CE). The red wines evaluated were found to range in tannin concentration from 33 mg/L CE (Lemberger) to 1071 mg/L CE (Cabernet Sauvingnon), with a mean tannin concentration of 589 mg/L CE. For the sensory evaluation study, Cabernet Sauvignon wines representing each tannin grouping were selected and panelists evaluated astringency using a 15-cm unstructured line scale. Sensory results indicated that panelists could distinguish between wines in the high and low tannin groupings ($p < 0.05$). These results indicated the relationship between analytical tannin values in Washington State red wines and their sensory perception of astringency.

Effect of Basic Tastes and Fat Content on Flavor Attributes of White Wine by Descriptive Analysis

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Our goal was to investigate how basic tastes (sweetness, sourness, saltiness, and umami) as well as oiliness in a food product affects wine flavor when consumed sequentially. Twenty formulations of "bread dips" varying in fat content (canola oil), salt (table salt with iodine), sugar (cane sugar), acid (citric acid), and umami (monosodium glutamate) flavored with a standard quantity of Dijon mustard and rice wine vinegar were created to fit a five-factor fractional factorial design. A descriptive analysis panel (n = 11) trained through consensus rated intensities of the sensory attributes of six white wines (Viognier, Sauvignon blanc, unoaked Chardonnay, oaked Chardonnay, dry Riesling, and an off-dry Riesling). Subsequently, panelists rated the perceived intensities of the wines after consuming bread (white sandwich) dipped in the specified bread dip. Finally, panelists again evaluated the wines without the bread dips. Results indicated all the attributes, except citrus aroma, differed among wines, bread dips, and/or wines interacting with bread dips. In general, there were some differences in the perceived attributes

of the wines tasted initially (without dips) and finally without dips. Most of these differences were due to changes in the panel's changing perceptions of the Viognier and the oaked Chardonnay over time. The presence of bread dips only affected the perceptions of pear aroma, oak aroma, sweetness, bitterness, tropical fruit flavor, and citrus flavor. In all cases the effects were significant but small (usually less than 1 unit on a 10-unit scale). These results are similar to those obtained with wine and cheese.

Rapid Prediction of Tannin and Anthocyanin Concentration in Red Wine Fermentations from UV-Visible Spectra

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The critical role of phenolic compounds in wine sensory properties (color and mouthfeel) and therefore perceived quality makes the development of simple, real-time tools to follow their extraction during fermentation an urgent need. At present, the most widely applied and established methods used to quantitate color and tannin in red wine extraction in both winery and research settings rely on colorimetric and chromatographic techniques and can require significant time for sample preparation and analysis. In the past decade, infrared spectroscopy has been examined for the development of predictive methods to measure a variety of grape and wine parameters. To date, researchers have used visible and near infrared spectroscopy with reasonable success to measure pH, sugar, alcohol, organic acids, anthocyanin, tannin, and polymeric pigments. However, limitations in this technology exist both in instrumentation costs and in the resources needed to prepare and validate adequate predictive models. At present, predictive methods exploiting information from the UV region of the spectrum have not been reported. In this work, partial least squares regression was applied to the UV-visible spectra of juices, fermentation samples, and finished wines. Analysis of data collected in California and Australia suggests that information in the UV-visible spectrum alone is able to quantify not only anthocyanins but also large polymeric pigments, total phenols, and tannin fractions as they are extracted from skins and seeds. Data demonstrating the potential of UV-visible spectra to predict quality parameters in red wines, musts, and juices will be presented.

Essential Growth Requirements of Malolactic Bacteria

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Lactic acid bacteria may positively contribute to wine quality as part of spontaneous or controlled malolactic fermentations. However, they may also lead to the production of compounds with negative health effects or to complete quality degradation of wines. The study of the pertinent metabolic activities is difficult because of the complex nature of the medium wine. For example, amino acids, whose metabolism may lead to the formation of biogenic amines and precursors of carcinogenic ethyl carbamate, may be released from proteins by proteases

during malolactic fermentations, and hydrolysis of glycosides can cause liberation of sugars hindering the calculation of substrate turnover, metabolic kinetics, and the determination of true growth requirements. In this study essential growth requirements of various oenococci and lactobacilli were investigated during the development of a minimal chemically defined medium by the single omission technique. The oenococcal strains were found to require more amino acids but the lactobacilli studied required more vitamins. Several compounds were required for growth by all strains tested. The results suggest that, similar to dairy lactic acid bacteria, wine bacteria exhibit complex and strain specific nutritional requirements. The study provides opportunities for the investigation of the bacterial metabolism and nutritional requirements of bacteria in wines. Well-formulated nutrient preparations could be used to favor bacterial genera, species, or strains selectively, in order to offer organisms with positive qualities an ecological advantage while reducing the likelihood of bacterial spoilage and the risk of formation of compounds with potentially negative health impacts, such as biogenic amines.

Impact of Thiamine and Pyridoxine on Wine Fermentation

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Sluggish fermentation and hydrogen sulfide production are serious problems found in wine industry. The objective of this study was to determine how different levels of thiamine, pyridoxine, and nitrogen affect yeast growth, fermentation rate, and hydrogen sulfide production. Three fermentations were conducted: 2 × 3 factorial design with nitrogen (60 and 250 mg/L) and thiamine (0, 0.2, and 0.5 mg/L), and nitrogen (60 and 250 mg/L) and pyridoxine (0, 0.25, and 0.5 mg/L) as variables, as well as a 3 × 3 factorial design for comparing the effects of thiamine (0, 0.2, and 0.5 mg/L) and pyridoxine (0, 0.25, and 0.5 mg/L) at the low concentration of nitrogen. *Saccharomyces cerevisiae* UCD 522 was used and fermentations were conducted at 22°C. Synthetic grape juice was fermented and hydrogen sulfide trapped throughout the fermentation. Thiamine and pyridoxine affected hydrogen sulfide production and fermentation rate. At low levels of thiamine and pyridoxine, yeast exhibited slower growth and fermentation rate compared to the higher concentrations at both low and high nitrogen levels. Hydrogen sulfide production was highly influenced by nitrogen and significant differences existed among different thiamine and pyridoxine levels. By adjusting the concentration of thiamine, pyridoxine, and nitrogen, sluggish/stuck fermentation and excessive hydrogen sulfide production can be reduced.

Metabolic Engineering of *Saccharomyces cerevisiae* to Minimize the Production of Ethyl Carbamate in Wine

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Saccharomyces cerevisiae metabolizes arginine, one of the major amino acids in grape musts, to ornithine and urea during wine fermentations. Wine yeast strains of *S. cerevisiae* do not fully metabolize urea during grape must fermentation. Urea is secreted by yeast cells and it reacts spontaneously with ethanol in wine to form ethyl carbamate, a potential carcinogenic agent for humans. The lack of urea catabolism by yeast in wine may be ascribed to the transcriptional repression of the *DUR1,2* gene by good nitrogen sources present in the grape must. We expressed the *DUR1,2* gene under control of the *S. cerevisiae PGK1* promoter and terminator signals and integrated this *DUR1,2* expression cassette, flanked by ura3 sequences, into the *URA3*-locus of the industrial wine yeast UC Davis 522 and the brandy yeast 228. *In vivo* assays showed that the metabolically engineered industrial strain reduced ethyl carbamate in Chardonnay wine by 89.1%; the brandy yeast reduced ethyl carbamate by 83.5%. Analyses of the genotype, phenotype, and transcriptome revealed that the engineered yeasts are substantially equivalent to the parental strains. The urea-degrading yeast, Davis 522EC$^-$, has received Generally Regarded As Safe (GRAS) status from the US FDA.

Effect of Microoxygenation Dosage and Oak Chips on the Tannic Properties of Spanish Red Wine

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The aging of a wine depends on its capacity to tolerate oxidation. This capacity is defined by the phenolic characteristics, their quantity, and the equilibrium among the different components. The enological characteristics of the wine (acidity, pH, alcohol content) and the phenolic components will determine the organoleptic wine properties. Microoxygenation is a technique employed by wine producers at different times of the vinification process in order to improve wine characteristics. At present, the addition of oak wood pieces (chips or sticks) in order to transmit characters of oak wood to the wine in combination with microoxygenation represents an interesting technique as a substitute to barrel aging. Modifications that affect the tannins present in the wine play a very important role in the final organoleptic characteristics of the wine. This work investigated the changes in tannin properties during microoxygenation of the wine aged with different oak chips. The variables analyzed were total polyphenols, low and high polymerized polyphenols, total tannins, catechins, total anthocyanins, gallic acid, HCl index, ethanol index, and gelatin index. The changes in wine phenolic compounds were similar in the different aging systems studied.

Analytical and Sensory Testing of Wines Made Using Crossflow Filtration

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Sensory and analytical testing were conducted on red, white, and rosé wines to determine if there were significant differences in aroma, flavor, and/or palate structure between conventionally filtered, crossflow-filtered, and unfiltered controls. Red and white wines from 2002 and 2003 vintages were sourced from cooperating wineries in California and the East Coast and evaluated at California State University, Fresno Viticulture and Enology Research Center laboratories. For sensory testing, a total of 20 triangle tests were conducted by a trained panel on 10 sets of wines: (a) five wineries supplied sets of unfiltered, conventional perpendicular flow (pad)-filtered and tangential-flow (crossflow)-filtered wines, (b) three wineries supplied only pad-filtered and crossflow-filtered samples, and (c) two wineries supplied only unfiltered and crossflow-filtered samples. Only one white wine among the eight pad-filtered versus crossflow-filtered wines (three white, five red) differed significantly (at 95% confidence level). Two of the seven pairs of unfiltered versus crossflow-filtered wines (one white, six red) differed significantly. The wines that had significant differences were a white and a red. One red wine among the five pairs of unfiltered versus pad-filtered wines (one white, four red)

differed significantly. The same wines were analyzed for total phenols (GAE mg/L). Results of the analytical testing yielded no significant difference in phenol concentration among the samples.

Ammonium Addition during Fermentation by Wine Yeasts: Effect of Quantity and Timing of Addition

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The objective of this work was to evaluate the relevance of nitrogen addition at two different times on deficient grape must during fermentation by two wine yeasts from Lamothe-Abiet. The must, from the Sauvignon variety, was diluted to 120 mg/L assimilable nitrogen and then supplemented with thiamine and sugars (50% glucose and 50% fructose) up to 240 g/L. For the two yeast strains, two assimilable nitrogen levels were studied: 190 and 240 mg/L. For each level ammonium sulfate was added in two ways: 100% before must inoculation or 50% before inoculation and 50% at the third day of fermentation (yeast growth phase). The growth and fermentation kinetics were determined as well as the consumption of assimilable nitrogen. The yeast growth improved when all nitrogen was added at the beginning and for the highest total concentration. The same observation was made for sugar consumption, except for one strain at 190 mg/L. Glucose was always exhausted and the residual sugar was only fructose. The presence of ammonium influenced only fructose consumption. In all cases the consumption of assimilable nitrogen was complete at any concentration or time of addition. Results suggest that the addition of ammonium salts in winemaking does not need to be done at several time intervals if the quantity added is not excessive.

Evaluation of Releasable 2,4,6-Trichloroanisole Tests for Natural Corks and Relationship with TCA in Bottled Wine

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The measurement of releasable 2,4,6-trichloroanisole (RTCA) in bulk cork soaks is now the main quality control used for detecting TCA in corks imported for the U.S. wine market. Little information is available to the wine industry for interpreting the results from these tests. We selected 10 bales of natural corks, imported from Portugal into the United States by several suppliers. For each bale, RTCA was measured from 10 group soaks of 50 corks each and from 100 individual corks. Replicates of group soaks evaluated the precision of this test and allowed grading the cork bales from low to high RTCA. The 1,000 individual cork soaks described RTCA distribution patterns within each bale, showing that bales with higher group-soak scores had more individual corks with relatively high RTCA. A bottling trial was performed with more corks taken from the same 10 bales. After

nine months, 100 bottles for each of the bales were opened and TCA measured in wine. Bales with higher RTCA scores showed higher percentages of bottles containing TCA of possible, or probable, sensory significance. Bales with lower RTCA scores showed the lowest percentages of those bottles, or none at all. This study confirms that RTCA is a good predictor of TCA transfer from natural corks to bottled wine, at least within several months. It also demonstrates that large-scale quality-control programs using RTCA tests with group soaks can reduce the occurrence of TCA-affected bottles in the marketplace.

Use of Oxygen Sensors to Nondestructively Measure the Dissolved Oxygen Content in Microoxygenated Wines

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Microoxygenation is used in several periods of wine elaboration to improve wine characteristics. Considering the rise of this technique, it is necessary to know the precise oxygen dosage as the concentration of solved oxygen in wine. Electrochemical sensors based on the Clark electrode provide a convenient tool for easy and rapid oxygen determination. However, the drift of these sensors prevents their use for accurate long-term measurements. On the contrary, optical sensing, particularly when combined with time-resolved fluorescence, could be a solution to get a long-term-stable oxygen sensor to nondestructively assess. The sensing principle is based on the quenching of fluorescence by oxygen. In this work the results of dissolved oxygen measurements to several microoxygenated aged wines from different origins are presented. The ability of optical oxygen sensors to monitor the levels of oxygen in the microoxygenation techniques was investigated with the classical electrochemical methods. The results and reliability of every employed technique have been studied and compared. The optical oxygen sensor system was shown to provide valuable information about the evolution of dissolved oxygen in red wine aging process in a convenient, nondestructive, and cost-efficient fashion.

Effect of Bottle Bore on Oxygen Transmission Rates of Natural Cork

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The oxygen transmission rate of natural cork closures is a primary factor affecting wine shelf life. The influence of bottle bore on the oxygen transmission rate of relatively high-grade cork closures was investigated. Ten A-grade natural corks nominally measuring 45 x 24 mm were tested for oxygen transmission rate in three sets of precision glass sleeves having internal diameters of 17.5, 19.5, and 21.5 mm. The mean oxygen transmission rate as well as the oxygen transmission rate distributions, significantly decreased with decreasing sleeve diameters.

Comparison of Aroma Compounds in Pinot noir Wines with Different Grape Maturity by Stir Bar Sorptive Extraction–Gas Chromatography-Mass Spectrometry

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A sensitive method using stir bar sorptive extraction gas–chromatography-mass spectrometry was developed to quantify the aroma compounds in wine. Calibration curves were built using five internal standards and pure standards in a synthetic wine matrix. The correlation coefficient (>0.95) and RSD (<10%) of the calibration curves demonstrate that this method could accurately measure volatile compounds that have been reported as important in wine. Two vintages of Pinot noir wines, with three different grape maturities each, were analyzed by this method. Statistical analysis showed that grape harvest maturity and growing year significantly affected the final wine aroma. Wine samples from a single harvest year indicate that grape maturity could affect compounds in different ways, based on their biochemical formation in the wines. For most grape-derived aroma compounds (C13 norisoprenoids and monoterpenes), concentrations increased with grape maturity in the final wine. Two oak-related aroma compounds, guaiacol and 4-ethylguaiacol, have increasing trends in Pinot noir wine samples. However, linalool showed the opposite trend, due to acid rearrangement during winemaking. Although there is no obvious trend for most fermentation-related aroma, some important characteristic esters for Pinot noir, including ethyl cinnamate, ethyl dihydroxycinnamate, and ethyl anthranilate, consistently decreased with grape maturity.

Efficacy of Rinse Procedures for Reducing Astringency Carry-over in Red Wine upon Repeated Ingestion

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Astringency has been described as one of the most important sensory attributes of red wine. An important attribute of astringency is that it has a temporal component and changes with time. Astringency demonstrates a carry-over effect, which translates to a changing perception of astringency over time. The objective of this study was to determine the most effective rinse to reduce the perception of astringency following repeated ingestions of red wine. During training, 18 panelists were trained to recognize basic tastes and astringency. Using computerized time-intensity (TI) methology, the effect of six successive ingestions of red wine was examined, with one rinse applied before to ingestion 1 and after ingestion 3. Following ingestion, a time interval of 40 sec was used for evaluation time, with expectoration at 10 sec. Rinses were applied for 25 sec, with a 40-sec interval between rinse and resumption of ingestion. The four rinses examined were deionzed water, pectin (1 g/L), carboxymethylcellulose (1g/L), and unsalted crackers. Data were analyzed by analysis of variance, a TI-averaging method and principal components analysis. Upon repeated ingestion, maximum intensity and time to maxi-

mum intensity were found to increase ($p < 0.05$) with successive ingestions. Cracker was found to be more effective at decreasing perceived astringency than pectin and carboxymethylcellulose, with water being the least effect at reducing astringency ($p < 0.05$). The identification of appropriate rinses over repeated ingestion is important as it is often within these circumstances that sensory panels or winetasting situations are conducted

Glycerol Oxidation in Wine and Reactions with Flavonoids

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Wine oxidation seems to include the formation of an especially reactive and thus nonselective oxidative compound, namely hydroxyl radical. This radical might be involved in the oxidation of important wine constituents and the production of a number of aldehydes and ketones. In this experiment, we studied the hydroxyl radical oxidation of glycerol, a major wine component, and thus a likely target of such oxidation, in model wine, generated by hydrogen peroxide and iron catalysis. The oxidation products generated were derivatized with 2,4-dinitrophenylhydrazine and analyzed as their hydrazones using liquid chromatography diode array and mass spectrometry. Among others, glyceraldehyde and dihydroxyacetone were the main compounds identified, both of which were also observed in naturally aged and hydroxyl radical oxidized wines. As anticipated, the presence of a high concentration of ethanol in the model wine did not preclude the formation of these compounds. Additionally, when a young red wine was treated with these oxidation derivatives, a significant increase in color was observed, most likely due to the formation of novel anthocyanin-based structures. The structures appear to be condensation products between the new electrophiles and flavonoids.

Determining Oxygen Ingress Quantities and Rates during Bottling and Storage in Any Package

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It is widely accepted that oxygen is a key reactant in the aging and flavor development of wine in its package. We can then assume that the amount and rate of oxygen incorporated into the package will have an affect on the order in which the oxidizable components in the wine are consumed. Oxygen is added to the wine during filling, from the headspace, during closure application and via ingress through the package during storage. There are benefits of some corrective measures and specific closure and packaging tests can be used to select the specific package (closure, liner, bag or plastic bottle) to give the long-term wine development or preservation desired. Data collected from bottling lines and package testing show that all similar corks, closures, liners, and plastic bottles do not perform the same.

Improvements in Measurement of pH and Titratable Acidity

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pH and titratable acidity (TA) are measured on grape juices, musts, and wines at various stages of preparation. pH is measured by placing a glass electrode directly into the undiluted sample and reading the result on a pH meter. TA is measured using a pH meter and manual burette or more commonly using an automatic titrator which carefully adds NaOH titrant to a diluted sample, all the time monitoring the pH until the typical endpoint of pH 8.2 is reached. Both measurements are easy in principle. However, problems can occur during repeated operation of the apparatus when large numbers of samples are analyzed sequentially. We have developed a single instrument for measuring pH and then TA of the same sample. The instrument uses automation to improve the analysis. Samples of 10 mL volume are pipetted into glass vials which are placed in a 50-position autosampler. The instrument moves the pH electrode, overhead stirrer, quartz dispenser probe, and temperature probe between buffer solutions, wash solutions, and sample, such that no human handling is required. This automation brings significant improvements to the accuracy and reproducibility of pH measurement. During the development of this new instrument, we investigated automated techniques for de-gassing samples on the instrument, washing probes in flowing water between samples to eliminate carry-over, and cleaning probes to remove protein deposits. The effectiveness of these automated procedures and their effects on the accuracy and repeatability of measurement are shown.

Oxygen Diffusion through Different Closures Used in Wine Bottle Aging: Effect of Storage Position

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Oxygen, one of the most important factors determining the aging potential and oxygen diffusion of/into bottled wine, is extremely dependent on the sealing effectiveness of the closure. A colorimetric method was set up to measure oxygen diffusion from 0.25 to 2.5 mL during the postbottling period. It is based upon the colorimetric measurement (L*, a*, b*) by direct scanning of commercial glass bottles containing indigo carmine solutions. This method was used to study oxygen diffusion through different closures available on the enology market. After 20 and 24 months of vertical and horizontal storage, only the control (all in glass bottle model) was impermeable to atmospheric oxygen. All other closures displayed variable rates of oxygen diffusion, which were much greater in the first month than in following months. The rate of diffusion was clearly influenced by the type of closure material used and was independent of the storage position for most of the closures, at least after 20 months of experiment. Oxygen diffusion rates were lower in technical/agglomerate corks, intermediate in conventional natural cork closures, and higher in the synthetic closures. Recent findings showed that over 5 months the screwcaps allowed an oxygen diffusion rate similar to the agglomerate/technical corks.

Alcohol in Beverage Measurement Using a Portable Variable Filter Array Mid-Infrared Spectrometer

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Measuring % alcohol by traditional methods can be a lengthy process limited to technically trained personnel in a laboratory. Typically, infrared analysis of alcohol is often measured in the near-infrared range (NIR). Mid-infrared (mid-IR), on the other hand, offers more distinct absorbance bands without the overtones found in the NIR range. Mid-IR also allow for the use of an ATR (attenuated total reflection) sample. The exposed ATR crystal makes sample presentation and cleaning rapid and easy. New advances in filter and detector technology have allowed variable filter arrays, which can give spectral information much like an FTIR yet in a compact, rugged, and less expensive package. Combined with a simplified PC interface, nontechnically trained personnel can obtain a measurement in less than a minute inside or outside the laboratory. Alcohol has an infrared absorbance in the mid-IR range at 9.54 microns or 1045 cm^{-1}. The absorbance increases and decreases with concentration which can then be correlated to a volume/volume measurement for % alcohol. With an elongated ATR crystal, an effective path lengh of 17 microns can be achieved. The measurement range with this path length is 2% to 95% alcohol with a +0.15% accuracy.

Trace Element Analysis by Means of Total Reflection X-Ray Fluorescence Applied to Quality and Origin Control in Wines

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Although the analysis of organic compounds and related ingredients is the main focus in wine analysis, the determination of inorganic trace elements is of critical importance as well. Several inorganic elements have a major influence on the quality of the wine and their concentrations must be tightly controlled. Wine producers must also comply with legal limits for inorganic elements, as defined by food, wine, or export regulations. An additional, increasingly important quality measure is origin and falsification control of wines by means of their trace element distribution. Total reflection X-ray fluorescence (TXRF) is an analytical method designed for the measurement of trace element concentrations. It combines simple sample preparation and quantification with high sensitivity and linearity. It is therefore a very suitable method for the determination of trace elements in wines. The possibilities and limitations for the application of TXRF to wine analysis are presented on the basis of recently completed measurements and areas for future development are discussed.

Soil Variability, Anthropic Changes, and Soil Management in a Napa Valley Vineyard

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Soils in a 4.5 ha Napa Valley vineyard were investigated to obtain a realistic large-scale map of soil variability. Natural soil variability, or soil profile changes resulting from cultivation, has an important impact on crop ripening and yield. The study site is on a complex convexo-concave slope cutting across a thick series of estuarine river alluvium with contrasted granulometry. Soil types include Haploxeralfs, Xerorthents, and Haploxererts, depending on underlying parent material, position on the slope, and recent cultivation practices. A preliminary survey included 44 auger holes regularly spaced on the slope, divided into six toposequences. Subsequently, 12 soil pits, forming three toposequences, were described and sampled based on preliminary observations. Each sampling point was precisely georeferenced using a GPS, and a digital elevation model was constructed using the elevation data. The soil samples were analyzed for soil texture, cation exchange capacity, organic matter content, and exchangeable cations. This information was then combined in a geodatabase with field observations such as coarse fragment content, soil color, and horizon thickness and analyzed using a geostatistical package. The lateral continuity of horizons, the presence of abrupt change thresholds, and gradients were examined. The optimal sampling density to conserve the greatest amount of information on soil characteristics was estimated by varying the number of observations included in the study. The study site showed a great parent material variability that required a larger number of sampling points than expected. Cultivation and grading before planting masked natural variability in the surface horizon.

Prediction of Grapevine Leaf Water Potential Using a Portable Near-Infrared Spectrometer

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Leaf water potential is a measure commonly used to describe grapevine water status and water stress dynamics. The established method for determining leaf water potential using a pressure chamber is cumbersome and subject to time/temperature limitations. These limitations prohibit the intensive sampling required to support proactive water management in commercial vineyards. As such, there is a need for faster, more precise, and more reliable tools for determining the leaf water potential of grapevines. To address this issue, near-infrared (NIR) spectroscopy and multivariate data analysis were applied for the modeling and determination of leaf water potential measurements using a pressure chamber as the reference methodology. Field trials using a portable NIR system showed correlation coefficients of 0.87 and 0.95. Analysis of the NIR spectra consistently demonstrated that grapevine leaf tissue had significant and intensive absorptions in the region from 1440 to 1950 nm, a spectral band known to indicate water in other

plant tissues. Leaf water potential determinations can be made in under 11 seconds using NIR, allowing for rapid assessments of water status across large vineyards. As water management becomes an increasingly critical component of vineyard management, NIR spectroscopy in conjunction with other current technologies such as geographic information systems can support better management, maximizing yield and quality.

Effects of Vineyard Floor Management Practices on Soil Physical, Nutritional, and Microbiological Characteristics

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A multidisciplinary trial evaluating the impact of vineyard floor management practices was conducted from 2001 to 2005 in Greenfield, CA. Using a split-block design, three weed-control strategies were assigned to the vine rows: (1) preemergence: simazine + oxyfluorfen followed by postemergence: glyphosate + oxyfluorfen; (2) postemergence only: glyphosate + oxyfluorfen; and (3) cultivation only. Three cover-crop strategies were assigned to the row middles: (1) bare, (2) Merced rye, and (3) Trios 102. Soil compaction was measured in the vine row in the early winter each year from 2003 to 2005. There was greater soil compaction in the cultivation treatment at the 4 to 8 inch depth in 2004 and 2005, which correlates with the depth that the blade of the cultivator passes through the soil. Water stable aggregates were measured in the vine rows and row middles in winter each year from 2003 to 2005. There were no differences among weed-control treatments but there was greater aggregate stability in the cover-cropped row middles in 2005. Plant and soil tissue samples were collected at flowering from 2003 to 2005. Weed control treatments did not affect the nutritional status of the vines. The greatest differences in soil nutrition were observed in the row middles: sodium, nitrate, and phosphorus were lower in the cover-crop treatments while soil organic matter was higher. Fourteen microbial biomass evaluations were conducted from winter 2001 to winter 2005. Weed treatment did not impact soil microbial biomass in the vine rows, but cover crops consistently increased microbial biomass in the row middles.

Role of Carbohydrate Nutrition in Bud Fruitfulness, Flower Cluster Necrosis, Berry Set, and Fruit Development of Stenospermocarpic Cultivars

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Adequate carbohydrate nutrition is necessary for normal bud fruitfulness and fruit development and to limit disorders such as coulure and flower cluster necrosis. Girdling increases carbohydrates (sugars and starches) in vine parts above the girdle. Princess and Summer Royal are two new USDA table grape cultivars. Like Thompson Seedless, they set by stenospermocarpy and berries contain only rudiments of seeds. Princess and Summer Royal can set poorly, resulting in

excessive numbers of unmarketable, poorly filled clusters (coulure). Flower cluster necrosis is a serious disorder with Princess. Girdles were applied to the trunk temporally, from early bloom through veraison, to diagnostically evaluate vine responses to pulses of increased carbohydrate nutrition. Flower cluster necrosis of the Princess cultivar was markedly reduced by the early bloom and full bloom girdles, with a 57% and 31% increase in harvested clusters, respectively. The full bloom girdle increased Princess berry set with a 38% increase in berries per cluster. Summer Royal berry set was increased by both the early bloom and full bloom girdles, with a 36% and 16% increase in berries, respectively. Berry weight for all three cultivars was increased from girdles applied during the period from full bloom until berry set, with the peak response occurring at the start of shatter. Girdles applied at veraison advanced maturity for all cultivars. Time required for the girdle wound to heal was five weeks and was similar for all three cultivars.

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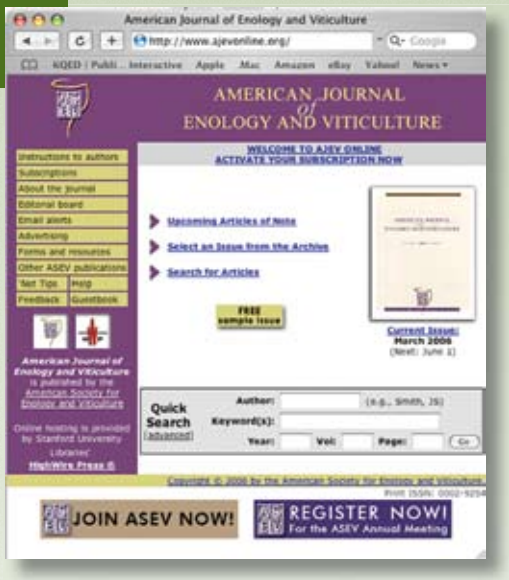

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