



52<sup>nd</sup> Annual Meeting

# Technical Abstracts

Oral and Poster  
Presentations

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# Alphabetical Listing of Presenting Authors

These abstracts in approximate order of presentation  
as noted in our conference program. There are no abstracts for  
the Experimental Design Workshop.

\*indicates Poster Presentation

Agosin, E. ....	43	Kupina, S. ....	*54
Agüero, C. ....	*74, *75	López, I. ....	*65
Anderson, M. ....	11, 31	Lu, J. ....	7
Bates, T. ....	18	Main, G. ....	*81
Bettiga, L. ....	*76	Marchal, R. ....	*45
Biscay, P. ....	*77	Mateus, N. ....	*55
Block, D. ....	42	Matsui, Hiroyuki ....	*82
Butzke, C. ....	1	Matthews, M. ....	39
Carrau, F. ....	*50	Mira de Orduña, R. ....	30
Celotti, E. ....	*51	Mlikota Gabler, F. ....	6
Ciana, M. ....	*62	Nail, W. ....	*72
Cocolin, L. ....	32	Navarro, L. ....	*68
Cohen, R. ....	8	Okamoto, G. ....	37
Colova-Tsolova, V. ....	24	Orr, E. ....	*66
Colugnati, G. ....	*78	Parr, W. ....	*56
Cousins, P. ....	*79	Peacock, W. ....	*83
Couto, J. ....	*63	Picciotto, E. ....	20
Dokoozlian, N.K. ....	17	Platt, J. ....	*46
Du, G. ....	34	Puech, J. L. ....	*57
Fenchak, S. ....	*52	Renaudin, R. ....	*58
Friend, A. ....	16	Rogers, B. ....	*86
Galzignato, D. ....	*69	Rogerson, F. ....	*59, *60
Gensler, W. ....	*80	Roush, T. ....	*73
Glynn, M. ....	9	Saenz, J. ....	14
Gomes de Pina, C. ....	*64	Sato, M. ....	*61
Guedes de Pinho, P. ....	*70	Shearer, D. ....	33
Gump, B. ....	4	Shore, F. ....	*47
Guo, X. ....	*87	Silva Ferreira, A. ....	2
Harbertson, J. ....	10	Sitton, G. ....	12
Hartmann, P. ....	3	Smart, D. ....	*84, 85
Hashim, J. ....	19	Smithyman, R. ....	38
Hetz, U. ....	26	Sommer, P. ....	*67
Howe, J. ....	35, 36	Swan, J. ....	*71
Iandolino, A. ....	40	Tat, L. ....	*48, *49
Julien, A. ....	41	Threlfall, R. ....	25
Keightley, K. ....	15	Van Vuuren, H. ....	44
Kilmartin, P. ....	22	Vasconcelos, M. ....	29
Kirrane, M. ....	*53	Walter-Peterson, H. ....	27
Kocsis, L. ....	21, *88	Wehmeier, G. ....	5
Kovacs, L. ....	23	Wolf, T. ....	13
Krivanek, A. ....	28		

# Monitoring of Wine Heat Exposure during Commercial Shipments

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The exposure of wine to elevated temperatures has a profound impact on its aging reactions and its sensory quality. This project monitored the heat exposure of wine during a significantly large and representative number of commercial shipments from winery to distributors across the United States under extreme conditions. Bottles containing model wine solutions and equipped with temperature data loggers were placed in different positions within a shipping container and accompanied the commercial freight. The results document the consequences of wine shipments in regular non-refrigerated trucks with different types of insulation. During the months of summer and early fall 2000, wines shipped to or via hot geographic locations were frequently exposed to temperatures above 75°F, and often for extended periods of time. Under the most extreme shipping conditions wines would have been exposed to temperatures above 110°F. A substantial daily fluctuation and a large variation in temperatures within one shipment were observed based on the position of the wine case within the container or truck. The accumulated heat exposure was calculated, and different kinetic models for wine aging were applied to make a comparison to wine storage under empirically ideal cellar conditions. These calculations suggest that the wines were exposed to heat during shipment that represented a corresponding aging time equivalent to between one month and six years. While the aging reactions between the various components of a wine vary substantially, different kinetics models may be used to better predict wine aging and bottle variation under different shipping and storage conditions.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Role of Some Technological Parameters as pH, SO<sub>2</sub>, O<sub>2</sub> and Temperature on the Formation of Some "Off-flavors" in White Wines

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Oxidative reactions in white wine may lead to drastic deterioration of wine in short periods of time. From the aromatic point of view this phenomena is characterized by the presence of certain off-flavors currently described as "potato", "paper", "honey", "green apple", "farm feed", "petroleum". Some of these off-flavors are associated to the presence of compounds such as methional, *trans*-2-nonenal, 3-octenol, ethanal and many others not yet identified. Certain technological factors, namely storage temperature, free SO<sub>2</sub>, dissolved O<sub>2</sub> and pH may influence the formation of these molecules in the wine. A lab scale experiment was carried out in which wines were submitted to different regimes of aging abuse (45°C temperature, O<sub>2</sub> saturation) during a storage period of 3 months. Samples were taken every 15 days for both sensorial and chemical analysis (GC – FD/FPD/MS, potentiometry - redox titrations, OD 280 and 420 nm). The results obtained show that terpenic alcohols and norisoprenoides such as β-ionone, β-damascenone and vitispirane which impart floral aromas to the wine decreased with temperature while the off-flavors described above arise being possibly related to the presence of ethyl phenylacetate, methional, mercaptoethanol, *trans*-2-nonenal and 3-octenol. The combination temperature/O<sub>2</sub> had a great affect on wine leading to a fast production of these compounds while the combination temperature/SO<sub>2</sub> led to the slowest. It was also observed that pH4 retarded the degradation of wine when compared to pH3.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# The Effect of Wine Matrix Ingredients on 3-Alkyl-2-Methoxypyrazine Measurement by Headspace Solid-phase Microextraction

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A systematic investigation of the effect of wine matrix ingredients and conditions on the headspace sampling of 3-alkyl-2-methoxypyrazines was performed with solid-phase microextraction and capillary gas chromatography using a nitrogen phosphorus detector. Changes in the recovery of 3-ethyl-, isopropyl-, sec-butyl-, and isobutyl-2-methoxypyrazines from the static headspace of synthetic wine matrices were studied and reported as function of SPME fiber type, extraction time, temperature, sample volume, and desorption temperature. The influence of added salt type and concentrations, pH, ethanol, gallic acid, and catechin were also studied. Oak and light exposure were subsequently tested to develop an understanding of factors affecting the concentrations of these compounds during processing and aging. The most effective and consistent analyses were achieved using DVB/Carboxen/PDMS and PDMS SPME fibers at 35 and 50°C for 30 minutes with 30% added sodium chloride. Sample volume did not exert an important influence on detection limit. Sodium chloride was the most effective salting agent of six salts evaluated. Ethanol increasing from 0 to 20% imparted an exponential decrease in the recovery of the analytes. Acidity affected a complete elimination of the analytes in the headspace at pH levels below 2. No significant impact on alkylmethoxypyrazine concentrations was observed with short exposures to gallic acid or catechin.

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# Comparison of Analytical Procedures for FAN in Grape Musts and Wine

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Yeast fermentable nitrogen in grape must and juice is present as *alpha*-amino acids and ammonium salts. Many processing decisions have an impact on the levels of FAN leading to deficiency. FAN deficiency in fermenting juice/must is often corrected by the addition of diammonium phosphate (DAP) and/or one or more commercially available nutrient supplements. The Formol titration is used worldwide as a simple and rapid determination of FAN. The method is simple to run and provides an approximate, but useful, index of the nutritional status of must/juice. The more recent NOPA procedure is also widely used to obtain similar nutritional information. A modification of NOPA allows one to focus specifically on arginine (ARGOPA), quantitatively the most significant contributor in yeast nutrition and to potential ethyl carbamate formation. These methods were utilized during the 1999/2000 harvest seasons on over 300 juice samples and the results were compared. Parallel recovery studies using model solutions of various amino acids present singly, and in combination, were also conducted. In that one of the major concerns relative to the use of the Formol method is formaldehyde, work directed towards reducing sample and reagent volumes was also performed. The Formol results do trend significantly with NOPA. ARGOPA results are consistently higher than either NOPA or Formol results. Adjustment of formaldehyde pH was critical to consistency of the Formol method. Ammonium nitrogen is also titrated in the Formol method and is obtained by a subsequent enzyme analysis in the NOPA method.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Use of Decision Tree Analysis in Evaluating the Influence of Viticultural Practices on Wine Chemistry**

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The purpose of this research was to use Decision Tree Analysis to examine the cause and effect relationship between variables in the vineyard and characteristics in the resulting wine. To this end, 36 individual lots of wine were produced from different existing vineyard trials. All trials utilized Cabernet Sauvignon grapes from the UC Davis Oakville Experimental Vineyard. Variables examined in the vineyard included vine spacing, trellising, pruning levels, irrigation levels, and rootstocks. All grapes were harvested at approximately 24 degrees Brix and fermented uniformly in order to minimize the influence of winemaking. Chemical characteristics of the resulting juices and wines were analyzed, including ammonia and  $\alpha$ -amino nitrogen (in juices), titratable acidity, pH, organic acids (by HPLC), phenolic profile (by HPLC), anthocyanins and color (by modified Somers method), and tannins (by Adams assay). Decision Tree Analysis was used to identify the variables in the vineyard that were most influential in determining each particular chemical characteristic. Continuing work on this project will use Decision Tree Analysis to identify variables in the vineyard that influence sensory characteristics as well.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Association of Anatomical and Chemical Characteristics of Mature Grape Berries with Their Resistance to Gray Mold

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Postharvest gray mold resistance of mature berries of 102 grape varieties and selections from the USDA-ARS vineyard in Fresno was assessed from 1997 to 2000. Spores of *Botrytis cinerea* were applied at four concentrations to detached berries, which were placed in humid boxes and stored six days at 15°C. The number of infected berries was counted, and the number of spores that caused half of the berries to become infected (ED-50) was calculated. Grapes were divided into four classes of resistance to gray mold (susceptible, slightly resistant, moderately resistant, and highly resistant) and resistant ones re-tested in following years. Little or no resistance existed in most popular *Vitis vinifera* varieties with the exception of the moderately resistant Emperor and Autumn Black. Highly resistant grapes were *V. labrusca* or *V. vinifera* x *labrusca* hybrids. In 2000, we began to examine which of the characteristics of grape berries of various levels of gray mold resistance were consistently associated with resistance to gray mold. The following chemical and anatomical features among 26 grape selections were quantified: 1) number of pores and lenticels per skin surface unit; 2) thickness of the skin and number of layers of specific skin cells; 3) skin protein content; and 4) skin total phenol content before and after *B. cinerea* infection. The association of these attributes with gray mold resistance was analyzed using bivariate correlation analysis. Few or no pores on the berry surface were consistently and significantly ( $P = 0.0006$ ) associated with resistance to gray mold. The number of lenticels per surface unit, number of skin cell layers, skin thickness, total protein content, and total phenol content were not associated with gray mold resistance.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **ABA Changes in Response to Pierce's Disease of Grapevines**

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Abscisic acid (ABA) is an important signal in triggering plant responses to environmental stresses caused by drought, salt, mechanical and pathogenic damage. As a means of studying the relationship between ABA content and Pierce's disease (PD), ABA were measured in healthy and diseased leaves, shoots and petioles of eight grape cultivars, including the *Vitis vinifera* grapes Chardonnay, Cabernet Sauvignon, and Thompson Seedless, the *V. labrusca* grapes Concord and Niagara, the Florida hybrid bunch grapes Blanc du Bois and Lake Emerald, and *V. rotundifolia* (muscadine grapes). Shoots with PD symptoms had 50 to 300% higher ABA concentrations than did healthy ones. The elevation of ABA content was consistently observed in xylem sap, and in petiole and leaf tissues of diseased branches. The ABA content elevation was smaller in the PD-resistant muscadine grapes than in the PD-susceptible bunch grapes. For both healthy and diseased leaves, the petioles had 2- to 6-fold higher ABA content than the leaf tissues among the cultivars investigated. The increase of the ABA content was apparently not contributed by the PD bacterium (*Xylella fastidiosa*) as no ABA was detected in *Xylella* cultures.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Evaluating the Effect of Rootstock on the Susceptibility of Chardonnay to Pierce's Disease Expression

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Pierce's Disease (PD), caused by the bacterium *Xylella fastidiosa* (Xf), is a lethal disease of grapevines that destroyed most of southern California viticulture in the late 19<sup>th</sup> century and continues to affect California's grape industry. Currently, there are no effective control strategies for PD. This experiment was designed to determine if rootstocks could effect the timing and severity of PD expression in 'Chardonnay' scions. 'Chardonnay' was grafted onto 18 commercially available rootstocks and onto five *V. vinifera* cultivars selected for their varying degrees of susceptibility to Xf. Ungrafted 'Chardonnay' vines and 'Chardonnay' grafted onto 'Chardonnay' vines were propagated as controls. Eighteen vines (three 6-vine replicates) of each treatment were tested under greenhouse conditions in a randomized complete block design. Partially lignified 50cm long shoots were inoculated with Xf about 20 cm above the soil level using the pin-prick method. One vine per treatment per replicate was inoculated with water as a control. The vines were assessed for PD leaf symptoms, including leaf scorching and senescence using a 0-5 rating scale (0=no symptoms, 5=dead vine). ELISA samples were collected 10cm above and 10cm below the point of inoculation four months after inoculation to determine the relative Xf concentrations at these locations. None of the rootstocks alleviated PD symptom expression. Correlations between Xf concentrations, leaf symptoms, and rootstocks will be presented.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Spatial Distribution of Brix, Phenolic Compounds, Berry Weight and Seed Number within a Cabernet Sauvignon Cluster**

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A mature Cabernet Sauvignon cluster was harvested from a Napa Valley vineyard and a three-dimensional chart was created to link each berry to its specific location in the cluster and relative solar orientation. Each of the 123 berries was numbered, weighed and analyzed for Brix, phenolic compounds and seed number. The skin from each berry was separated from the pulp and seeds. Using the juice from each berry, the Brix was determined by refractometry. The skins received individual phenol extractions through a procedure which involved an initial mincing (using scalpels and a crucible), followed by a heated extraction in an acidified ethanol solvent. The individual extracts were analyzed using reversed phase high-pressure liquid chromatography. A diode array detector measured absorbance at four different wavelengths which will allow us to calculate relative quantities of catechins, hydroxycinnamates, flavonols and anthocyanins. The HPLC analysis has been performed but the data has not been analyzed. To facilitate graphical representation, a two-dimensional map of the cluster has been created and will be used to display the variation of each compound measured. The average Brix per berry was 22.1 and the standard deviation = 0.9. In addition to overall spatial variation, the berries were placed in two groups based on their solar exposure (north or south). Although the comparison of the northern and southern berry groups showed insignificant differences in mean Brix and standard deviation, the total berry weight was 82% greater in the southern berry group. The attached chart displays the relationship between Brix, berry weight, and seeds per berry.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Analysis of Polymeric Pigments during Berry Development, Fermentation, and Wine Aging**

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It is thought that the polymeric pigments in wines are formed by reaction of monomeric anthocyanins with polymeric flavan-3-ols. We previously reported that polymeric pigments could be separated into two distinct classes; Large Polymeric Pigments (LPP), which bind to proteins and precipitate along with tannins, and Small Polymeric Pigments (SPP) which remain in solution after the tannin and LPP have been removed by precipitation. In order to determine which of the wine color components are present in fruit, we assayed the amount of each one weekly from veraison to harvest in skins of Cabernet Sauvignon and Syrah. In wines, LPP typically accounts for 30% of the total color at pH4.9, but in skins of both varieties less than 10% of the color was due to LPP. This suggests that LPP is largely formed during winemaking. The SPP accounted for a large percentage of the color in grape skins as well as in finished wines. This suggests that SPP is extracted from skins along with monomeric anthocyanins during fermentation. To test this premise we monitored color components during a commercial scale Syrah fermentation. The amount of LPP, SPP and monomeric anthocyanins increased during winemaking, and in the finished wine LPP accounted for a much larger percentage of the color than it did in the fruit. In order to determine how polymeric pigments changed during aging we studied 16 Cabernet Sauvignon vintages ranging from 1973 to 1998, made with fruit from the same vineyard. Results show that the percentage of color due to SPP varied from year to year, but did not show a clear trend with aging. However, the percentage of color contributed by LPP increased during wine aging, with a concomitant decrease in the percentage of monomeric anthocyanin.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Influence of Rootstock on Vine Mineral Nutrition Status

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The utility of grape rootstocks is far from being limited to their original mission of protection from soil borne pests such as phylloxera and nematodes. This study investigates the influence of rootstocks on vine nutrient status and is part of a decade-long investigation of the influences of rootstocks on vine performance. To assess vine nutrient status, tissue samples were collected from three winegrape rootstock trials. All three trials included the same 14 rootstocks and the data reported are the means of four years. Two trials were located in the Sacramento River Delta area, one with Chardonnay and one with Cabernet Sauvignon as scions. The third trial was located in the Shenandoah Valley of Amador County and the scion was Zinfandel. The tissue samples were analyzed for total K (% dry weight) and total N (% dry weight) and  $\text{NO}_3^-$  (ppm). Rootstocks had a significant influence on the levels of all measured elements at all sites. Rootstock influence resulted in 2.9, 2.5 and 2.2x differences in bloom petiole K at the Delta Chardonnay, Delta Cabernet Sauvignon and Shenandoah Valley sites, respectively. Data for bloom petiole K was greatest for the rootstock 44-53 Mgt at both Delta sites and for Freedom at the Shenandoah Valley site. Rootstocks with low levels of petiole K were 110R at the Delta Chardonnay site and 420A at both the Delta Cabernet Sauvignon and Shenandoah Valley site. The influence of rootstock on bloom petiole  $\text{NO}_3^-$  was greatest at the Delta Chardonnay site where there was a 5.5x difference between the high (1103P) and low (1616C) rootstocks. The rootstock 420A had the lowest bloom petiole  $\text{NO}_3^-$  levels, 2.9 and 4.9 times lower than the high rootstocks 5BB and Ramsey at both the Delta Cabernet Sauvignon and Shenandoah Valley sites respectively. Bloom petiole N was lowest at all sites for 44-53 Mgt.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **The Roles of Skins and Seeds in Phenolic Extraction during Alcoholic Fermentation and Extended Maceration**

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The individual roles of skins and seeds, in the phenolic extraction from berries, during alcoholic fermentation and extended maceration, is not well characterized. This is due to the difficulty associated with separating skins and seeds. This experiment divides a single vineyard lot of Sonoma Valley Cabernet Sauvignon into four half-ton treatments at day four: an early press (no skins or seeds), an early press with the seeds separated and returned to the juice, an early press with the skins separated and returned to the juice, and a normal extended maceration. The four treatments were performed in duplicate. The mean number of seeds, per berry, taken prior to crush, and again after the treatments indicate a separation of better than 75%. Juice samples were taken daily from crush, through fermentation, and through a 17-day extended maceration. Samples are currently being analyzed by reversed-phase HPLC to profile the phenolic extraction over time. Further, samples are being analyzed by spectrophotometer to determine the role of tannin concentration on observed color, due to copigmentation. The Adams-Harbertson assay will be used to quantify the tannin content of the samples. The tannin assay quantifies tannin polymers of four subunits or greater. Monomeric tannin from HPLC analysis will be correlated with tannin polymers from the Adams-Harbertson assay to characterize tannin complexing during fermentation and extended maceration.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Response of Shiraz Grapes and Grapevines to Five Different Training Systems in the Barossa Valley

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Own-rooted Shiraz, spaced 1.5 m apart in 2.75-m wide rows, were compared under five different training systems at an Orlando-Wyndham vineyard in the Barossa Valley, South Australia. The objective was to determine how training affected fruit quality and crop yield in the context of promoting efficient vineyard management. Training systems were: single wire (SW), vines were trained to bilateral cordons, 1.0 m above the ground, non-shoot-positioned; high single wire (HSW), a higher cordon (1.8-m) version of SW; vertical shoot positioned (VSP); Scott-Henry (S-H), canopies of alternate vines were shoot-positioned upwards or downwards; and minimally pruned (MP), same as SW, except vines were not annually spur-pruned. Crop yields (kg/m of row), since 1996, averaged 3.69 (VSP), 5.48 (MP), 3.18 (SW), 3.24 (HSW), and 4.04 (S-H). Focusing on the 1999-2000 season, crop yields were similar for VSP, MP, and S-H vines (3.2 to 3.7 kg/m of row), which in turn were greater than HSW or SW crops (2.5 to 2.7 kg/m of row). For S-H vines, crop per m of canopy of downward-oriented canopies was roughly 50% (1.0 kg/m) of upward-oriented canopies (2.2 kg/m), due to lower clusters per shoot (0.8 vs. 1.2) and lower shoot counts per vine (70 vs. 83). Fruit of the HSW, SW, MP, and the upward-trained canopy of S-H vines all showed similar rates of sugar accumulation during the 1999-2000 growing season, while VSP and the downward-trained canopies of S-H vines were slightly retarded. Fruit from the two canopies of S-H ultimately ripened to ca. 24.5°Brix; however, fruit from the lower canopy was 0.1 pH units higher than that (pH 3.55) from the upper canopy at harvest. Fruit anthocyanin and total phenolics concentrations (mg/g berry fresh wt.) at harvest were least with VSP-trained vines, and comparable among other treatments. Looking at all treatments, fruit anthocyanin and total phenolics concentrations exhibited a negative relationship with crop/m of canopy, and a slight positive relationship with cluster exposure. The SW, HSW, and MP training systems all provided good yields of high quality fruit, although MP did have a tendency to overcrop in some years. VSP and S-H training were less attractive due to greater canopy management requirements.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Pruning Grapevines Modifies the Economy of the Whole Plant Mineral Nutrition

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The traditional winter pruning technique removes more than 70% of the canes. This removal drastically reduces the number of buds, the yield potential, and a significant portion of whole-vine mineral and energy reserves. The objective of this study was to assess the effects of pruning on whole-vine nutrient content, distribution, and uptake from pre-bloom to harvest. Two treatments were used: one was severely pruned to only 40 nodes per vine; the other was minimally pruned, removing only the end of the cane that was in a 50 cm space above the ground. The first important difference found was that minimal pruning significantly increased the number of buds/vine, number of shoots/vine, number of clusters/vine and the yield. In addition, minimal pruning resulted in almost no reduction in the amount of mineral nutrients, compared to the substantial reduction of mineral nutrients on 40-node pruned vines. Moreover, severe (40-node pruned) pruning stimulated stem growth in direct relationship to its intensity. It is clear, therefore, that severe pruning creates an unproductive cycle of mineral nutrient allocation into stems, which are later removed by pruning. Given that the size of the plant biomass largely controls the size of the nutrient pool reserve, and that minimally pruned vines have larger over-wintering biomass, an increased pool of mineral nutrients is available to them early in the spring. In addition, the larger woody biomass of minimally pruned vines displayed lower nutrient concentrations for N, K, and P, especially at pre-bloom, suggesting that a larger amount of nutrients are being mobilized for re-use in current growth. These many features indicate consistency of mechanisms, favoring an expeditious canopy development in minimally pruned vines. Early canopy development is not only suggestive of increased photosynthesis early in the season, but also of increased transpiration. This implies an increased soil nutrient uptake by mass flow early in the season.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Influence of Row Orientation and Cluster Exposure to Sunlight on the Microclimate and Composition of Cabernet Sauvignon Fruits in the Napa Valley**

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Interactions between row orientation and cluster sunlight exposure on fruit microclimate and composition of Cabernet Sauvignon (*Vitis vinifera* L.) were examined in a commercial vineyard near Oakville, CA. Selected clusters were exposed to a range of sunlight exposures in two orthogonal row orientations. Exposed clusters received up to 400 times more midday solar radiation than the most shaded clusters. This variability in solar exposure resulted in differences in fruit temperature of up to 10 °C, with exposed fruit reaching over 40 °C. Light environment varied appreciably between the two sides of the hedgerow and between the two row orientations. Vines in an east-west row orientation intercepted radiation primarily on the south side of the canopy, which established large cumulative differences in microclimate in the fruit zone on the two sides of the canopy. Vines in the north-south row orientation intercepted radiation in more equivalent amounts in the fruit zone on each side of the canopy due to morning insolation on the east side of the canopy and afternoon insolation on the west side of the canopy. Average soluble solids were greater ( $p \leq 0.01$ ) for fruit grown in the east-west oriented row. Titratable acidity, pH, malate, phenols and anthocyanins showed no significant variation between rows. Brix, malate, phenols and anthocyanins exhibited significant mean differences between the two sides of the rows ( $p \leq 0.01$ , malate  $p \leq 0.05$ ). Titratable acidity and pH showed no significant variation between row sides. Cluster exposure categories reveal trends in fruit composition with increasing solar exposure. Brix values increased ( $p \leq 0.01$ ) with higher solar exposure, while malate and juice pH declined ( $p \leq 0.01$ ). Berry size and color were negatively impacted by high radiation exposure with significant variation correlated with row orientation.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Manipulating Bud Break Date in Grapevines

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Spring frost is a significant hazard in many cool climate grape-growing regions. Damage to vegetative parts of the grapevine occurs when ice crystals are formed, disrupting plant cells. Pre-bud break, super-cooling prevents water from forming ice crystals, and as a result grapevines can tolerate significant cold events (down to  $-20^{\circ}\text{C}$ ). As buds start to grow, xylem differentiation occurs in the developing shoot, creating possible ice nucleation sites. As a consequence, the ability of plant tissue to undergo super-cooling is removed and damage may occur at temperatures just below freezing. The probability of freezing temperatures occurring decreases as spring progresses. Therefore, cultural methods that delay the onset of bud break will decrease the risk of frost damage. Grapevines exhibit strong apical dominance. Delaying winter pruning until after bud break of apical buds delays the onset of basal bud development. This potentially provides greater frost tolerance. Application of an alginate gel to grapevine canes creates a similar delay in basal bud break. An experiment was established to evaluate the response of bud break to delayed pruning and alginate gel application. A  $-1.5^{\circ}\text{C}$  frost event occurred at the onset of bud break. The additive effect of pruning and the alginate gel resulted in a delay of bud phenological development, and coincided with a reduction in the incidence of frost damage to primary shoots. Secondary buds emerged from nodes where primary buds were frost damaged. The development of secondary shoots has implications for increased variation in fruit and wine quality. Delayed bud break has also been found to result in substantial yield increases. Yield increases arise from higher average bunch weights and larger berries. It is theorised that delaying bud burst may improve the fertilisation rate of ovules within flowers. The improved fertilisation rate increases the seed mass within berries, which in turn increases average berry weight.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Influence of Forchlorfenuron (CPPU) on the Growth and Composition of Table Grapes**

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Several studies were conducted between 1998 and 2000 to determine the effects of the synthetic cytokinin forchlorfenuron (CPPU) on the fruit growth and composition of several table grape cultivars, including Thompson Seedless, Flame Seedless, Ruby Seedless, Melissa and Redglobe. CPPU (0,3,6,9 or 12 mg/L) was applied to mature vines either alone or in combination with 20 to 40 mg/L gibberellic acid ( $GA_3$ ), at fruit set. In some experiments CPPU was also applied to vines at veraison to determine effects on fruit ripening rate and composition. CPPU applied at fruit set resulted in maximum berry weight increases between 11% (Redglobe) and 40% (Thompson Seedless) compared to the untreated controls. Across all cultivars the mean berry weight of vines receiving CPPU +  $GA_3$  at berry set was approximately 16% greater than vines receiving either material alone. In Thompson Seedless, Ruby Seedless and Melissa berry diameter increased as the concentration of CPPU applied at fruit set increased, while berry length reached its maximum when vines were treated with 6 mg/L CPPU. The combined mean berry length:diameter ratios for CPPU and  $GA_3$  treated fruits of these cultivars was 1.45 and 1.65, respectively, indicating that CPPU applications resulted in a more spherical or round berry shape compared to  $GA_3$  applied alone. CPPU applied at fruit softening had no significant effect on berry growth. CPPU applied at both berry set and berry softening delayed fruit maturation. A two-week delay in harvest of most cultivars was obtained when 9 to 12 mg/L CPPU was applied at berry set, while pigment accumulation was either delayed or significantly reduced in all red-colored cultivars. CPPU had no significant effect on vine yield components or on subsequent vine fruitfulness the year following its application.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **The Effect of Fruit Thinning on Yield, Quality, and Return Crop in 'Concord'**

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For 'Concord' growers in the Northeast, US, conservative pruning limits the yield potential in above average growing years. In contrast, excessive bud numbers can lead to an overcrop in poor growing years. The practice of leaving more buds for maximum crop and fruit thinning in poor ripening years is increasing in New York vineyards. The objective of this research is to measure the physiological response of 'Concord' to crop adjustment 30 days after bloom. 120 node (44 buds per meter of row) hand pruned 'Concord' grapevines were hand thinned in 10% crop increments from 100% crop to 0% crop. From a yield equivalent of 24.8-9.0 tonnes/hectare (11-4 tons/acre), there was an inverse relationship between yield and juice soluble solids, juice color, and ripe nodes of periderm. In year two, the vines were again pruned to 120 nodes. There was a direct relationship between ripe nodes of periderm in year one and yield in year two. These relationships did not continue at thinning levels below 9.0 tonnes/hectare (4 tons/acre). Additional 120 node vines were mechanically thinned with two different thinning machines and two thinning rates. Moderate machine thinning was identical to hand thinning in the parameters measured. Severe machine thinning damaged shoots and led to lower fruit maturity at a comparable hand thinned treatment. This research shows the positive response of 'Concord' grapevines to fruit thinning 30 days after bloom and indicates that moderate machine thinning is similar to hand thinning.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Interaction between the Leaf Area:Fruit Weight Ratio and Cultural Practices on Three Table Grape Cultivars in California**

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A four x two factorial experiment was designed to examine the interaction between leaf area (main plots) and table grape cultural practices (sub-plots) on the fruit growth, color development and composition of three principal California table grape cultivars: Flame Seedless, Redglobe (seeded) and Thompson Seedless. The trial was conducted in 1999 at the UC Kearney Agricultural Center in Parlier, CA. The effects of leaf area were examined by defoliation of whole vines at berry set to approximately 25%, 50% and 75% of the undefoliated control vines. Within each defoliation treatment (main plots), two sub-treatments were used to examine the effects of cultural practices on fruit growth and composition. These consisted of gibberellic acid applications to reduce berry set (seedless cultivars only) and increase berry size, as well as a girdle to increase berry size. Flame Seedless and Thompson Seedless treated with gibberellic acid and girdled had similar leaf area:fruit weight requirements, approximately 8 cm<sup>2</sup>/g to 11 cm<sup>2</sup>/g, while Redglobe required approximately 5 cm<sup>2</sup>/g to 7 cm<sup>2</sup>/g, for optimum fruit size and composition. At the same leaf area:fruit weight ratio, cultural practices consistently produced larger Flame Seedless and Thompson Seedless fruit, and reduced the soluble solids and anthocyanins at harvest, compared to untreated fruit. Defoliation treatments did not affect the number or weight of seeds in Redglobe berries. The data indicates that modern table grape cultural practices have direct physiological effects on fruit growth and composition apart from those that can be attributed to concomitant reductions in the leaf area:fruit weight ratio.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **The Effects of Bisulfite Treatments and pH Changes on the Absorbance of Polymeric Pigments at 520 nm**

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Red wine color is due to free monomeric anthocyanins as well as polymeric pigments. As red wine ages the monomeric anthocyanins are progressively incorporated into polymers. This is thought to occur by reaction of monomeric anthocyanins with polymeric flavan-3-ols (tannins) to give pigments with a high molecular weight. After several years red wine color is due almost exclusively to the presence of polymeric pigments. Spectrophotometric determinations of polymeric pigments in the presence of anthocyanins typically involve bleaching anthocyanins with bisulfite and then considering the residual absorbance (520nm) to be due to polymeric pigments. In these kinds of analyses bisulfite bleaching of polymeric pigments is assumed to be negligible. We separated the polymeric pigments from a 1980 Cabernet Sauvignon wine into two fractions by chromatography on a Toyopearl HW-40(F) column and verified by HPLC that there were only traces of monomeric anthocyanin. We also showed the absence of monomeric anthocyanins by monitoring changes in absorbance (520nm) of the wine with changes in pH. Both polymeric pigment fractions from the Toyopearl column showed bleaching with bisulfite. We obtained additional evidence that polymeric pigments bleach with bisulfite using a protein precipitation assay that separates them into small polymeric pigments (SPP) that do not precipitate with protein and large ones (LPP) that do. From our results it appears that traditional color assays that rely on bisulfite bleaching overestimate the amount of monomeric anthocyanin and underestimate the amount of polymeric pigment. More accurate values for the components of wine color can be easily obtained if bisulfite bleaching of polymeric pigments is taken into account.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Grape Rootstock – Scion Combination Effects on Leaf Nutrient Status and Yield Under Drought Conditions in Hungary.**

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The influence of six rootstocks (8B, 5BB, 5C, GK28, 140 Ru and Fercal) on nutrient uptake, vegetative vigor, yield and fruit quality was tested under non-irrigated conditions with high ('Hungarian Riesling'), medium ('Italian Riesling') and low vigor ('Vinitor') *Vitis vinifera* cultivar rootstock – scion combinations. The plot was set up with four 10 vine blocks with the 18 treatment combinations. Data from an unusually dry season in the Lake Balaton region of Hungary, when 155 mm of rain fell between budbreak and harvest, were compared to mean data from three years. Statistically significant differences in N, P, K, Ca and Mg levels, yield and °B were found. Mineral uptake among the *V. berlandieri* X *V. riparia* based rootstocks (8A, 5BB, 5C and GK28) was similar, but were significantly different with 140Ru and Fercal, particularly in K, Ca and Mg. Large differences were observed in P content, but P levels were very low and the scion appeared to have the greatest effect on P level. Potassium levels were highest in the least vigorous scion ('Vinitor'). Yield was correlated with scion vigor, but differences in °B were not correlated. The interaction between rootstock and scion had the largest effect on nutrient uptake, and dry conditions also greatly altered nutrient uptake when compared to years of normal rainfall.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Cyclic Voltammetry Characterisation of Antioxidants in Wines

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A cyclic voltammetry method has been developed to quantify antioxidants in wines according to their reducing strength at a carbon electrode, the analysis taking about 3 min per sample. 10-fold dilution of white wines and 400-fold dilution of red wines in a model wine solution (12% ethanol, 0.033 M tartaric acid adjusted to pH 3.6) was required for reproducible data, with a first peak at 400 mV of 1-3 mA due to the oxidation of phenolic compounds with an ortho-diphenol group. A second peak or shoulder at 450 mV was ascribed to flavonol glycosides, as confirmed by simulated voltammograms constructed from wine phenolic composition and the cyclic voltammetry response of individual standards. A third peak at 620 mV, seen in red wines only, was ascribed to the malvidin anthocyanins, while further oxidation current beyond 700 mV was due to meta-diphenol or isolated phenol groups and other oxidisable compounds. The method separates phenolics by their relative ease of oxidation, and a measure of the lower oxidation potential phenolics (the more reactive antioxidants) was given by the integral of the current up to 500 mV, reported as gallic acid equivalents. A good correlation was obtained between this measure and the Folin-Ciocalteu and  $E_{280}$  values of the total phenols for 5 white wines. More variation was seen between the cyclic voltammetry and Folin-Ciocalteu measures for 6 red wines. Further investigation will reveal the full utility of this quick method which may be able to discriminate the phenolic content qualitatively.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# De-Acclimation of *Vitis amurensis* Hybrid Varieties in Fluctuating Winter Temperatures

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*Vitis amurensis*, a wild grape from Siberia, has been exploited as a genetic source for cold hardiness by numerous breeding programs in Europe and Asia. The usefulness of this species for North American viticulture is limited by its propensity to readily break dormancy in response to even a short period of mild temperature. This feature is undesirable under the North American continental climate that is characterized by large temperature fluctuations in winter. Although wild accessions and F1 hybrids are well known for their unstable dormancy, little is known about more advanced hybrids whose genome derives from *V. amurensis* in <12.5% percent, and whose parentage also includes American *Vitis* species. The purpose of this study was to examine the de-acclimation properties of such hybrids under continental climatic conditions. In this study, seven hybrid selections were planted with each selection replicated six times in a completely randomized block design. The experiment included the *V. amurensis*-*V. vinifera* F1 hybrid 'Michurinetz' and the complex *V. vinifera*-American hybrid 'Vignoles' as controls. A budbreak survey conducted during the 1999/2000 dormant season revealed that *V. amurensis* hybrids initiated bud growth four to five weeks earlier than 'Vignoles', with bud swell occurring as early as the first week of January. The early de-acclimation in these plants has been confirmed by the results of a second budbreak survey and a budbreak assay conducted during the 2000/2001 dormant season. In the budbreak assay, all *V. amurensis* hybrids significantly differed ( $P < 0.001$ ) from Vignoles in the number of days required for 50% of the buds to start at the beginning of dormancy (late October and November), but not in mid-dormancy (December). The differences in the de-acclimation patterns of the various *V. amurensis* hybrids decreased with the advance of the dormant season.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Genetically Engineered Grape for Seedless and Stress Tolerance

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DNA recombinant technology comprises the removal of specific DNA fragments from one organism, the study of their functions and their subsequent incorporation into existing genetic background, which may be a chosen plant species. Molecular breeding should not be assumed as a separate alternative of conventional plant breeding, but as a complementary approach at a later stage. The efficiency of a genetic transformation procedure depends on several factors: the right choice of the plasmid construct, optimal conditions for transformation, ability to regenerate plants from transformed tissue, proper and accurate selection and possibility of developing intact transgenic plants.

The crucial requirement in the process of transformation is the availability of cells having both the ability to be transformed and that to regenerate into plants subsequently. Thus, embryogenic cells in suspension are very promising candidates for successful gene transfer in grape. The present work was aimed at development of highly efficient technology for gene transfer in grape: enable to perform multiple transformations applicable not only for direct genetic improvement, but also to be efficient tool for basic study of the gene expression in grape. Two parallel protocols for initiation somatic embryogenesis (SE) respectively from in vivo anthers and in vitro young leaves were developed. For Muscadine grape (*Vitis rotundifolia*) cvs Fry and Triumph additional protocol was established using as a primary explant for SE in vivo petioles and anthers. Embryogenic lines in suspension culture have been successfully inoculated (*V. vinifera* cvs: Velika, Rusalka, Victoria, Cabernet, Merlot, Chardonnay, Flame Seedless; rootstock cvs: 110 Richter, Rupestris du Lot). A well working approach for monitoring of each developmental state of the process of SE has been used to provide the opportunity for extremely high regenerative ratio of the putative transgenic plants and proper genetic selection (selective agent equal to 100mg/L kanamycin). Agrobacterium-mediated gene transfer were performed in embryogenic suspension culture. Histochemical Gus assay and molecular analysis including PCR and Southern blot were used to confirm the 'transgene.' The traits of interest are seedless, disease (viral, bacterial, fungal) and cold tolerance, The following gene constructs have been utilized: SF4 - Barnase gene under the control of seed coat specific promoter, CP of GFLV, Sarcotoxin (lytic peptide), and three different constructs on a base of antifreezing gene isolated from Antarctic fish.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Effectiveness of Sulfur Dioxide and Dimethyldicarbonate on *Saccharomyces bayanus* Growth at Different pH Levels in a Juice and Semi-sweet Wine**

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Since many companies are interested in reducing filtering and the level of preservatives, a study was developed to evaluate the reduction of sulfur dioxide (SO<sub>2</sub>) in juice and wine through the use of dimethyldicarbonate (DMDC). SO<sub>2</sub>, DMDC, and their combinations were studied to evaluate their effectiveness on *Saccharomyces bayanus* growth at different pH levels in a semi-sweet wine. Two studies were created to determine quantitatively the yeast growth after inoculation (Study 1) and to monitor the time until visible fermentation after inoculation (Study 2). The treatment design contained three factors, SO<sub>2</sub> levels (0, 10, 25, and 50 mg/L), DMDC levels (0, 50, 100, and 200 mg/L), and pH levels (3.0, 3.2, 3.4, and 3.6). The two studies were done on both the juice and semi-sweet wine. The project was based on research published in the American Journal of Enology and Viticulture by Ough et al., 1988 and the Journal of Food Science by Terrell et. al., 1993. The juice and wine were inoculated with 500-700 colony forming units/mL. All treatments in the juice and wine with no preservatives showed significant yeast growth and fermentation. The treatments containing 50 mg/L SO<sub>2</sub> or 100 and 200 mg/L DMDC completely prevented yeast growth and fermentation in the wine. The 10 mg/L SO<sub>2</sub> treatment alone and the 50 mg/L DMDC treatment alone did not prevent yeast growth or fermentation at any pH level in the wine. The treatment with the least amount of preservatives that prevented yeast growth and fermentation in the wine was the combination of 10 mg/L SO<sub>2</sub> and 50 mg/L DMDC. Higher levels of preservatives were needed to prevent fermentation in the juice.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Lysozyme as an Aid in Preventing Stuck Fermentations

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The growth of certain lactic and acetic acid bacteria in the early stages of wine fermentation is known to be one of several causes of incomplete fermentations. We evaluated native hen egg white lysozyme (NL) and a partially unfolded variant (PUL) to inhibit the growth of these bacteria and thus avoid a cause of stuck fermentation. Lysozyme is normally active only against Gram-positive bacteria, however Ibrahim *et al.* have reported that lysozyme when partially unfolded by heating at 80° C will display non-enzymatic antimicrobial activity against Gram-negative bacteria such as *Escherichia coli*. In our study, *Lactobacillus kunkeei*, reported by Edwards *et al.* to cause stuck fermentations, was inhibited by using 300ppm NL lysozyme. A 10<sup>5</sup> CFU/mL inoculum in Chardonnay must was reduced to <10 CFU/mL within 48 hours while populations in the control treatment (no Lysozyme) reached 10<sup>7</sup> CFU/mL. *Acetobacter aceti*, shown by Drysdale and Fleet to lead to incomplete fermentations, was inhibited by a PUL lysozyme (PUL) but not by NL lysozyme. Initial bacterial populations (10<sup>6</sup> CFU/mL) of two different strains of *A. aceti* were reduced by more than 90% after a two-hour exposure period to 200ppm of PUL.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Screening of *Vitis rupestris* x *Muscadinia rotundifolia* Hybrids for Resistance to Grapevine Fanleaf Virus

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Grapevine fanleaf virus (GFLV) is one of the most serious viral diseases of grape. This virus is carried and spread in vineyards by the dagger nematode, *Xiphinema index*. Previous work has identified resistance to *X. index* feeding in *Vitis rupestris* X *Muscadinia rotundifolia* hybrids, and resulted in identification of a DNA marker that is tightly linked to dagger nematode resistance. In this study, we evaluated the GFLV resistance of *V. rupestris* x *M. rotundifolia* hybrid selections. Inoculation of the hybrids was accomplished by herbaceous approach-grafting rooted cuttings of the hybrid plants with rooted cuttings of GFLV-infected 'Cabernet Sauvignon'. The presence of GFLV was determined 5 months after inoculation with ELISA. The grafted plants were then cut, leaving the hybrid plant as the scion and the roots of the inoculum source as the rootstock, to evaluate graft success. Genotypes determined to be resistant will be used in inheritance studies with second generation sibling hybrid populations [(*V. rupestris* X *M. rotundifolia*) X (*V. rupestris* X *M. rotundifolia*)] to identify DNA markers associated with GFLV resistance and to determine the number and location of GFLV resistance genes.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Progress in the Genetic Analysis of Pierce's Disease Resistance

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Pierce's disease (PD), caused by the *Xylella fastidiosa* (Xf) bacterium, has the potential to devastate California's viticulture industry. Development of PD resistant varieties can provide a long-term solution to this disease, and molecular genetic techniques will help speed this process. Xf resistant selections derived from a *Vitis rupestris* 'A. de Serres' X *Muscadinia rotundifolia* 'Cowart' cross (8909) have been identified in a greenhouse screen based on cane symptoms and lack of bacteria movement. A Design II mating design with 6 females and 6 males has been created to produce the populations necessary for inheritance studies, to develop DNA markers for resistance, and to work towards identifying Xf resistance genes. The 8909-15 X 8909-17 cross, previously used to establish a mapping population for a *Xiphinema index* resistance gene, was screened for Xf resistance. Three replicated cuttings from 150 genotypes were grown under greenhouse conditions, inoculated with Xf bacteria and evaluated for resistance in the same manner as the parent population. About half of the family members have AFLP marker information and AFLP marker data is being generated for the remaining individuals. Additional mapping populations have been generated and will also be evaluated for Xf resistance. The goal of this research is to understand the number and location of genes that control Xf resistance. AFLP mapping of these populations will allow marker aided selection in the breeding program and allow efforts to identify and clone Xf resistance genes.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Evaluation of Eleven Chardonnay Clones in Oregon

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Yield components, pruning weights, and juice composition of 11 Chardonnay clones were measured for the 1996-2000 seasons in a replicated trial planted in 1989. Data from the 5-year evaluation was pooled. The clones included in the trial consisted of the Dijon (DJN) clones 75, 76, 78, 95, and 96; Espiguette (ESP) clone 352; and Foundation Plant Material Services (FPMS) clones 4, 5, 6, 14, and 15. Vines were balanced pruned during the 5-year study period. ESP 352, FPMS 6, and DJN 78 respectively, had the highest yield per vine compared to the other clones. FPMS 15 had the lowest yield per vine followed by FPMS 14 and DJN 95. FPMS 15, FPMS 6, and ESP 352 had the highest berry weight while FPMS 4, 5, and 14 had the highest average cluster weight. FPMS 15 and DJN 95 had the lowest cluster weight. FPMS 15 had the highest pH. FPMS 4, 5, and 6 had the lowest pH and the highest titratable acidity. DJN 95, 96, 75, respectively, had the lowest titratable acidity. DJN 76 had the highest soluble solid content and ESP 352 had the lowest. The Dijon clones 75, 76, 78, and 95 had moderate to high bud fertility (clusters/shoot), low to moderate berry weight, cluster weight, and percent fruit-set. DJN 96 performed similarly but had very low cluster weights compared to the other clones under evaluation. ESP 352 consistently had the highest bud fertility in addition to the highest yield while FPMS 4, 5, and 14 had a high percent fruit-set and very compact clusters in comparison to the other clones. Clones with a consistently high Ravaz index (yield to pruning ratio) were FPMS 14, 4 and 5. Clones with a consistently low Ravaz index included FPMS 15 and DJN 95.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Effect of Malolactic Strain and Timing of Inoculation on Chemical and Sensory Characteristics of Chardonnay

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The application of simultaneous alcoholic (AF) and malolactic fermentations (MLF) in the production of table wines can offer a number of microbiological, technical, and financial advantages. In this work, two *Oenococcus oeni* strains (LALVIN EQ54 and ALPHA) were used to induce MLF simultaneously with AF, or after AF by *Saccharomyces cerevisiae* strain LALVIN CY3079 in Chardonnay must from Hawke's Bay (NZ). The resulting wines were analyzed for their chemical composition and sensory characteristics. Wines made by consecutive AF/MLF had ~750 mg/L<sup>-1</sup> of combined glucose and fructose. Though, no glucose or fructose was measurable in wines produced by simultaneous AF/MLF, which were called "super-dry". Acetaldehyde concentrations in consecutive AF/MLF treatments reached higher maxima during fermentation (60-80 mg/L<sup>-1</sup> as compared to 40-50 mg/L<sup>-1</sup> for simultaneous AF/MLF) and were higher upon bottling (7-13 mg/L<sup>-1</sup> as compared to 2-8 mg/L<sup>-1</sup>). Contrary to reports about increased acetic acid concentrations coming from MLF in grape must, acetic acid concentrations were only marginally higher after simultaneous AF/MLF ( $\bar{O}$ =245 mg/L<sup>-1</sup> compared to  $\bar{O}$ =215 mg/L<sup>-1</sup> after consecutive AF/MLF). Sensory analysis by discrimination tests revealed no significant differences between the wines for any combination of treatments. Flavour profiling led to the aroma attributes "Fruity" and "Butter/Caramel" and the taste attribute "Acidity". Higher intensity ratings for "Fruity" were found in simultaneous treatments, whereas "Butter/Caramel" and "Acidity" were perceived more intensive in consecutive treatments. Besides the time advantage of the simultaneous AF/MLF-procedure, it may be generally possible to achieve wines with low hexose concentrations that are microbiologically more stable, allowing to reduce the use of SO<sub>2</sub>. The time course of acetaldehyde concentrations during simultaneous fermentations may influence red wine colour development. The kinetics of several other compounds was studied, as well.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Evaluation of Chardonnay Clones for Sparkling Wine Production

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This study evaluates 12 Chardonnay clones and one locally used field selection (“Wente”) for their viticultural and enological attributes for the production of base wines for sparkling wine production. The trial is located in the Carneros District of Sonoma County. The trial includes clones from California and France that are currently used for still wine production as well as clones recently imported from France, specifically selected for sparkling wine production. For several of these clones this trial represents their first appearance in a replicated trial in California. Data has been collected for 3 years (1998 – 2000); the data presented is the mean of these years. All clones were pruned to an equal number of buds. All clones were harvested on a Brix basis with consideration of acid levels. Harvest of all clones took place within 4 days except FPMS 4 that was harvested 9 days after the first clone. In all three years FPMS 4 was the last clone harvested. Mean harvest °Brix was 20.8 and, with the exception of the “Wente” clone, the maximum deviation from the mean was 0.3 °Brix. Mean yield for the plot was 5.7 kg vine<sup>-1</sup> with FPMS 4 (6.4 kg vine<sup>-1</sup>) and the “Wente” selection (3.4 kg vine<sup>-1</sup>) having the highest and lowest yields respectively. The only significant yield difference was between the “Wente” selection and the other 12 clones. Differences in yield were primarily due to differences in both berries per cluster and berry weight leading to differences in cluster weight. FPMS 4 had heavier clusters due to significantly more berries per cluster. The “Wente” selection had both the fewest berries per cluster and the lightest clusters.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Molecular Biology Methods as a Tool for Quality Control in Wine Fermentations. Application of DGGE, RFLP and mtDNA-REA to a Continuous Fermentation**

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The quality of wine is a direct consequence of the evolution of the microbial flora of the must during fermentation. The transformation of grape juice into wine is the result of a large variety of microorganisms. A wide range of yeast populations, including species of *Kloeckera*, *Metschnikowia*, *Candida*, *Hanseniaspora* and *Saccharomyces* are often present in the initial stages of most wine fermentation. While in the first stages of the fermentation the non-*Saccharomyces* strains dominate, stronger fermenting and more alcoholic-tolerant species of *Saccharomyces* take over and complete the fermentation.

Little information is available regarding the capability of *S. cerevisiae* to perform continuous wine fermentations. In this study, we report the results obtained using a new approach, based on molecular biology methods, to monitor a continuous fermentation. A continuous fermentor (TMCI Padovan S.p.a, Conegliano, Italy), inoculated with a selected *S. cerevisiae* strain and producing fermenting must at 5% alcoholic degrees, was followed for 15 days. Direct and indirect methods were used to determine if the inoculated strain was able to persist throughout the fermentation. Denaturing gradient gel electrophoresis (DGGE) on a 26S rRNA gene PCR product, restriction fragment length polymorphism (RFLP) on 18S rRNA gene PCR product and restriction enzyme analysis on mitochondrial DNA (mtDNA-REA) were performed.

The results obtained demonstrated the capability of the *S. cerevisiae* strain used to dominate the fermentation throughout the period followed. In addition, the use of the selected molecular methods proved to be a powerful tool in conducting quality control of ancient processes, such as wine fermentation.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Participatory On Farm Trials for Sustainable Viticulture

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The inherent variability of climate, soil and management between viticultural regions in Australia means that new viticultural practices require regional assessment and validation. Participatory On Farm Trials for Sustainable Viticulture introduces a model for improving viticultural management practices, by encouraging and supporting growers to test and validate research outcomes on their own vineyards. It seeks to enable and empower growers to test new viticultural practices and make informed decisions about vineyard management based on their trial results and those of others within their regions. The project takes an integrated approach to development, evaluation and adoption of sustainable vineyard management practices through collaboration between researchers from different disciplines and practicing viticulturists. It aims to help growers learn to define their research needs and assess sustainable viticultural practices on a regional basis through participatory on-farm trials. The project aims to increase the confidence, commitment and capacity of growers to critically select and implement sustainable viticultural practices. It also develops participating researcher's skills in identifying and developing new areas of research and implementing effective technology transfer.

A consultative process identifies grower and regional needs. Ensuing trial results provide relevant information flows throughout and beyond the regions. Researchers from different disciplines are directly involved in defining and testing key issues facing growers who are adopting new management practices. Through this process, regional acceptance and adoption of improved practices, is being increased and equally grower awareness of economic and environmental sustainability issues is heightened. The capacity of growers to adapt these practices to different conditions and circumstances will be enhanced by a broadening of their knowledge base through these trials.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Effect of Partial Rootzone Drying on Vine Water Relation, Vegetative Growth, Mineral Nutrition, Yield Components, Fruit Composition, and Wine Chemistry in Sauvignon blanc Grapevines**

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Partial rootzone drying (PRD), derived from split root research, is an irrigation technique which modifies vine growth and development by keeping part of the rootzone dry and the rest of the rootzone well-watered at a reduced irrigation rate. The feasibility and effect of PRD were studied in the San Joaquin Valley of California in 1999 and 2000. The research was conducted with bilateral cordon trained Sauvignon blanc/Freedom grapevines on Hanford Sandy Loam. Treatment factors included PRD and conventional drip irrigation (CDI) at 0.4 and 0.8 crop evapotranspiration, resulting in 4 treatments, PRD-0.4, PRD-0.8, CDI-0.4, and CDI-0.8. The vines were irrigated daily during the growing season and the treatments were applied between fruit set and harvest. The wetting and drying sides of PRD treated vines were alternated at an interval of 7-20 days. Total water usage of PRD-0.4 and CDI-0.4 was between 54-69% of CDI-0.8. Reduced irrigation rate at 0.4 ETc both in PRD-0.4 and CDI-0.4 resulted in a decrease in stomatal conductance, transpiration rate, vine vegetative growth, and water use efficiency. Light penetration into fruiting zone, petiole mineral nutrient contents, yield, fruit composition, and wine chemistry were not influenced by either irrigation methods or irrigation rate, except CDI-0.4 had higher Ca content. The study demonstrated that PRD offers a way for producing a vine with less vegetative growth while maintaining yield comparable to standard irrigation practices for high vigor vineyards. However, most of the observed effect on vine performance and vine physiology was resulted from the reduction of irrigation rate rather than drying part of the root system.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Leaf Gas Exchange and Pruning Weight Response of Pinot noir to Manipulation of Soil Moisture and Nitrogen Availability

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The goal of this study was to observe vine physiological response to various vineyard practices aimed at increasing nitrogen availability to the vine. Treatments were applied in a factorial design to vary irrigation, cultivation, and nitrogen application to Pinot noir grapevines during two seasons. Irrigated vines received 50mm supplemental irrigation after lag phase. Cultivation of alternate rows was performed in early spring to encourage nitrogen utilization and reduce nutrient and water competition. Nitrogen treatments consisted of soil-applied urea (35lbsN/acre), foliar applied urea (2.66lbsN/acre), and zero nitrogen. Soil nitrogen was applied in early spring. Foliar nitrogen was applied once at the beginning of véraison and again at 50% color change. The trial was established in a commercial vineyard located in the South Willamette Valley in 1999. Photosynthesis, transpiration, water use efficiency, chlorophyll content, maximum quantum yield of photosynthesis, and pruning weights of mature Pinot noir vines were measured in the 1999 and 2000 growing seasons. During both seasons irrigated vines assimilated CO<sub>2</sub> and transpired at a significantly higher rate than non-irrigated vines. Similarly, tilled treatments assimilated CO<sub>2</sub> at a significantly higher rate and maintained higher water use efficiency. Tilling tended to increase the efficiency of light driven photosynthetic reactions and chlorophyll content. This response became more apparent in the second year of the study, which may indicate a long-term effect of soil cultivation on maximum quantum yield of photosynthesis and chlorophyll content in grapevines. Nitrogen treatments had little impact on leaf gas exchange and chlorophyll content. Cultivation had the only significant treatment effect on pruning weight in 1999, while it had a significant effect on both pruning weight and cane weight in 2000.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Manipulation of Soil Moisture and Nitrogen Availability to Improve Juice Composition of Pinot noir Grown in Oregon

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The aim of this study was to optimize fruit quality and maximize the juice nitrogen fraction that can be used by yeast during fermentation. Various vineyard practices that may improve nitrogen availability to the vine were integrated into a replicated trial in a commercial vineyard located in the South Willamette Valley in 1999. Treatments were applied in a factorial design to vary irrigation, cultivation, and nitrogen application to Pinot noir grapevines during two seasons. Irrigated vines received 50mm supplemental irrigation after lag phase. Cultivation of alternate rows was performed in early spring to encourage nitrogen utilization and reduce nutrient and water competition. Nitrogen treatments consisted of soil-applied urea (35lbsN/acre), foliar applied urea (2.66lbsN/acre), and zero nitrogen. Soil nitrogen was applied in early spring. Foliar nitrogen was applied once at the beginning of véraison and again at 50% color change. Petiole nutrient content, ripening dynamics, juice composition, and yield components of mature Pinot noir vines were measured in the 1999 and 2000 growing seasons. Soil cultivation had the largest impact on petiole nutrient content, with significant differences in phosphorus, potassium, manganese, copper, boron, carbon and total nitrogen. Irrigation increased total petiole nitrogen in the second year of the study. During both seasons, irrigated vines had lower titratable acidity than non-irrigated vines and tilled treatments had higher soluble solids than non-tilled treatments. In 1999, the ammonia content decreased from an average of 80 to 30 mg (N/L) while the alpha amino acid content increased from 60 to 150mg (N/L) during ripening. The YANC increased from 100 to 190mg (N/L), predominately due to a large increase in alpha amino acid concentration in the last two weeks of maturation. The results from 2000 indicate that tilled treatments tend to have higher YANC at harvest, predominately due to a large increase in alpha amino acids. Significant effects from tilling suggest that there may be a delayed benefit to soil cultivation. Irrigated vines tended to have a higher berry weight, however, yield components differed only slightly between treatments and year.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Effect of Vine Water Stress on Berry Ripening in Chardonnay Grapes

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Chardonnay vines planted in root zone restricting beds were subjected to water deficit stress by withholding irrigation for a period starting 15 (early stress; ES) or 25 (late stress; LS) days after veraison until harvest. Control vines were provided with normal irrigation. Both the ES and LS treatments significantly decreased the leaf water potential and caused leaf wilting 2 days after the start of the treatment. The ABA level in the leaves around clusters increased significantly by both the ES and LS treatments. The ABA level in berries increased more rapidly by the ES treatment, though the level was not significantly different between the LS and control vines. Berry harvesting in ES vines was carried out 10 days after the start of the treatment, 10 days earlier than that in the LS and control vines, because of the severe berry shrinking and leaf fall. The levels of juice TSS and acidity were not affected significantly by both the ES and LS treatments. However, the ES treatment has resulted in a significant accumulation of amino acids such as PRO, ALA, GLN, ARG, and GABA during 10 days after the start of the treatment, though the total level of amino acids at harvest was highest in LS treated vines. Tasting tests of the wines produced from the berries of treated and control vines showed that the wine from LS vines was highest quality. This study reveals that the water deficit stress for 10 days before harvest increases the level of several major amino acids in Chardonnay grape berries and improves the wine quality.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Deficit Irrigation and Summer Hedging Influence on Canopy Growth and Fruit Quality of Riesling

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This research was initiated in 2000 in an effort to control canopy growth and improve fruit characteristics of Riesling through deficit irrigation management. The following treatments were imposed: 1) the standard practice of minimal irrigation during early-season to achieve desired shoot length and reduce shoot growth, then irrigation to replace vine water consumption for duration of season (ST); 2) an additional water deficit period during cell division in addition to the standard practice, then irrigation to replace vine water consumption for duration of season (ED); 3) irrigation throughout season to promote increased canopy growth (EI); and 4) irrigation throughout season with summer hedging to increase cluster exposure after veraison (EH). EI increased shoot length while ED reduced shoot length compared to ST. The EI and EH treatments increased berry weight whereas ED reduced berry weight compared to ST at harvest. Cluster weight and yield expressed the same relationship among treatments as found in berry weight. No differences in °Brix, titratable acidity, or pH were measured among treatments at harvest. Further research to determine phenolic development during fruit ripening and vinification is currently ongoing. By examining the influence of periods of water deficit stress and hedging during the growing season on the phenolic profile of fruit and wine, new management practices could be developed that have the potential for improving grapevine production and wine quality while conserving water resources.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Responses of Berry Size and of Tannins, Pigments, and Sensory Attributes in Cabernet Sauvignon Grapes and Wine to Water Deficits and Crop Load**

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The mechanism(s) by which differences in composition develop in differentially irrigated red winegrapes and their wines is not clear. Experiments were initiated to investigate how yield, berry growth, berry water status, and cluster microclimate participate in responses to water deficits. Vine water status in commercial vineyards was well separated by applying the standard rate of irrigation, withholding water, or applying 2x the standard rate. In separate experiments with no irrigation treatments, machine harvested berries were manually separated into three size classes; and, vines were pruned or cluster thinned to produce six yields ranging from 0.5 to more than 2x standard bud numbers per vine. Berry size was significantly reduced by withholding irrigation, but was similar between standard and 2x irrigation in 2 seasons. Berry size was not different among the 6 yields produced by pruning or cluster thinning. Fruit and wines were analyzed for color, acidity, phenolics, and sensory descriptive analysis. Small berry wine (SBW) and Medium berry wine (MBW) were very similar. For example, in both wines free monomeric pigments, polymeric pigments, and copigmentation accounted for 35%, 25%, 40% of the total color, respectively. However, Large berry wine (LBW) had a larger proportion of polymeric pigment (35%) and less copigmentation (27%) than wines made from smaller berries. Total phenolic concentration was significantly higher in SBW than in the other two treatments and was 20% greater than in the LBW. Wines from the irrigation and berry size experiments were evaluated by descriptive analysis. Differences were found among the low, standard, and 2x irrigation wines in the intensities of green beans aroma, red fruit by mouth, bitterness and astringency. Differences were also found among the SBW, MBW, and LBW in the sensory attributes linked to phenolic extraction, e.g., bitterness and astringency.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Effects of N Fertilizer and Irrigation Amounts on Leaf $\delta^{13}\text{C}$ Values, Seasonal Water Status, Gas Exchange and Water Use Efficiency of Cabernet Sauvignon Vines

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The effects of N fertilizer and irrigation amounts on leaf stable carbon isotope composition ( $\delta^{13}\text{C}$ ), seasonal water status, net  $\text{CO}_2$  assimilation ( $P_n$ ), water use efficiency (WUE) and biomass production were determined on Cabernet Sauvignon vines grafted onto the rootstock 110R. The experiment was conducted in a commercial vineyard near Oakville, California, during the 1999 growing season. The fertilizer treatments consisted of a control (no applied N) and vines receiving a single application of 35 and 71 kg N ha<sup>-1</sup> two weeks pre-bloom. The irrigation treatments consisted of various fractions (0.25, 0.5 and 1.0) of estimated, full vineyard water use. Seasonal, midday leaf water potential ( $\Psi_1$ ) was significantly increased at greater applied water amounts and lower fertilizer rates. The  $\delta^{13}\text{C}$ , the relative change in  $^{13}\text{C}/^{12}\text{C}$  isotopic molar ratio, of canopy leaves collected at leaf fall were significantly correlated with  $\Psi_1$ . Seasonal  $\Psi_1$  and  $\delta^{13}\text{C}$  were also significantly correlated with normalized, seasonal averages of leaf stomatal conductance ( $g_s$ ),  $P_n$  and transpiration rates (E). Both photosynthetic and canopy WUE showed significant correlations with  $\Psi_1$  and  $\delta^{13}\text{C}$ . Results indicate that  $\delta^{13}\text{C}$  could be used as a relative indicator of seasonal vine water status, water use efficiency and as an integrative index of vine stress.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Temperature and pH Effect on Yeast Strain Nitrogen and Oxygen Utilization

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In winemaking, oxygen and nitrogen are beginning to be recognized as two very important factors that play an essential role in the kinetics of alcoholic fermentation. Their deficiency in must is responsible for stuck or sluggish fermentations. This investigation shows not only variability in the requirements for these two nutrients between different yeast strains, but also the influence of temperature and pH on their uptake. We propose a method to quantify oxygen and nitrogen wine yeast strain requirements, and study the variability under different temperature and pH conditions.

Wine yeast strain nitrogen requirements, determined during the primary fermentation stationary phase, were measured as constant rate fermentations under three different temperatures and pH conditions. Significant differences in nitrogen uptake were observed between wine yeast strains under the same conditions. When the same wine yeast strain was tested under different temperature and pH conditions, we also noted significant differences in their nitrogen demand. Oxygen requirements of wine yeast strains were compared by running anaerobic fermentations and measuring the effect of adding 1 mg/L oxygen at the beginning of the stationary phase. The effectiveness of this oxygen addition under three different temperatures was different depending on the wine yeast strain. The temperature influence was verified in a Chardonnay grape juice.

These results allow the winemaker to make adjustments in the amounts of nitrogen and oxygen to add when managing fermentations under different temperature and pH conditions in order to limit the occurrence of stuck or sluggish fermentations.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Prediction and Prevention of Problem Wine Fermentations Using a Mechanistic Model

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A physical and mathematical model has been developed previously by our group to predict sugar and nitrogen utilization and biomass production given initial nitrogen and sugar levels in juice. The rate of fermentation, cell growth, cell viability, and ethanol production were examined. A correlation between the maximum viable biomass and the fermentation rate exists and this correlation may lead to a convenient method to predict fermentation problems within the first two days of the fermentation. The model prediction of transition from normal to sluggish to stuck fermentations with decrease in initial nitrogen level was verified experimentally. The model also predicts the ability to restart sluggish or stuck fermentation with the addition of nitrogen or actively fermenting cells. Based on this, low nitrogen fermentations were conducted and various forms of nitrogen and cells were fed to verify the model experimentally. In order to significantly reduce time to fermentation completion the addition should be made after the initiation of growth and prior to the fermentation reaching 5 °Brix. While a high percentage of cell viability was observed after sugar utilization ceased, a point existed in these fermentations at which the addition of nitrogen had no effect on metabolism, potentially due to the inability of the yeast to transport the added nutrients.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Modeling Yeast Metabolism and Process Dynamics in Wine Fermentation

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Wine fermentation is an inherently dynamic process in which the culture's environment varies over time. Hence, fermenting yeasts are subject to several different stress conditions that trigger specific regulatory mechanisms.

A dynamic stoichiometric model was developed for the simulation of the wine fermentation by cultures of *Saccharomyces cerevisiae*. The model is represented by a differential-algebraic system of equations. The set of differential equations describes the evolution of the environment conditions, switching from osmolarity to ethanol toxicity. The set of linear algebraic equations, on the other hand, describes all feasible metabolic pathways and reaction constraints, which are activated or deactivated depending on specific external and internal signals. Redox balance and growth rate as well are optimized using an appropriate objective function. The whole fermentation process is simulated as an iterative process, with an optimization routine applied each iteration. Growth rate, homeostasis and redox power coupling are considered as cellular objectives and maximum growth rate is determined for each iteration step according to nutrient availability and environmental metabolic requirements.

The influence of the environment on metabolic flux distribution and the capacity to link metabolic networks structure and flux bounding with environmental variables was successfully addressed by the model developed, reproducing experimental wine fermentation yields for different musts.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Genome-wide Expression of Genes in Wine Yeast during Chardonnay Fermentations: Regulatory Effect of Di-ammonium Phosphate

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The aromatic complexity of wines vary according to the primary or varietal aromas associated with the grape variety from which they were derived, the secondary aromas produced by yeast during fermentation, and the tertiary aromas that develop during ageing. The fermentation bouquet therefore plays an important role in the final aroma of wine. The production of secondary aroma or spoilage compounds by yeast can be significantly affected by winemaking practices such as clarification of grape musts, and the addition of yeast nutrients to fermenting grape musts. The nitrogen composition of grape musts affects the kinetics of fermentation, the production of aroma and spoilage compounds, and urea, a precursor of the carcinogen ethyl carbamate. Since the early eighties it has become a common practice in wineries to supplement grape musts with di-ammonium phosphate to prevent nitrogen-related fermentation problems. It is, however, well established that laboratory strains of *S. cerevisiae* preferentially use rich nitrogen sources, such as ammonia, over poor nitrogen sources. The addition of di-ammonium phosphate could, therefore, affect the expression of a large number of genes in wine yeasts. Recent development of techniques to monitor gene expression in *S. cerevisiae* on a genomic scale represents a huge step towards the application of functional genomics in industrial fermentations. We used the Affymetrix GeneChip Instrumentation system and custom GeneChip expression probe arrays to monitor gene expression patterns on a genomic scale in the industrial wine yeast strain Vin13 during vinification of Chardonnay grape must. We were able to score 4480 of the more than 6000 genes in *S. cerevisiae*. The addition of di-ammonium phosphate affected the expression of at least 386 genes more than two-fold; 259 were down-regulated and 127 were up-regulated. Di-ammonium phosphate regulated the expression of genes in pathways leading to the production of ethyl carbamate, fusel oils and H<sub>2</sub>S.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Wheat Gluten Proteins Used as a Clarifying Agent of Red Wines

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Bovine spongiform encephalopathy caused a situation of crisis leading the public and winemakers to lose their confidence in the use of gelatin as fining agent and to reject animal proteins in general. Therefore we started the research of a substitute for gelatin and egg proteins, by comparing gluten with these fining treatments currently used. This study concerned the fining of two red wines, a Pinot noir from Burgundy (Rully, Controlled Appellation) and a Gamay from Val de Loire (Gamay Touraine, Controlled Appellation).

Burgundy Pinot noir : For 6 g/hL, hydrolyzed glutens gave better efficiencies than deamidated glutens. The egg proteins treatment was situated between the hydrolyzed glutens and deamidated glutens. For 12 and 18 g/hL, turbidities of the wine treated by five glutens were 67 to 86% less than the control wine. Better results were obtained with egg proteins for short kinetics particularly. Wine fining with gluten was always better than gelatin treatments. The difference between the five glutens became very small when the dose incorporated in the wine increased. The volumes of lees generated by fining with gluten are situated between the values obtained with egg proteins and gelatin. After fining, immunodetection with gluten polyclonal antibodies showed a complete elimination of exogenous enological proteins.

Gamay Touraine : For all the glutens, egg proteins and the gelatins used at 12 g/hL, turbidities were situated between -73% and -77% of the non treated Gamay wine turbidity. Thermal treated gluten generated a volume of lees largely lower than the values observed for egg proteins and gelatin, the two actual animal fining agents used for red wines. When wines were compared using triangular tests, it was not possible to differentiate the control wine and the gluten treated wines.

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# A Classification Model of the Winemaker's *Vinifera* Grape Decision

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A winemaker has a fundamental choice as to the variety of wines to produce from *vinifera* grapes. While there is a multivariate set of earth science, climate, economic, and related factors that must be considered in constructing a reasonable portfolio of varieties, and while different winemakers place different weights on the relative importance of each of these and other considerations, ultimately the winemaker must make a yes/no decision with regard to each varietal wine derivative of *vinifera* grapes. The objective here is to construct classification models that predict the winemaker's decision with respect to several popular varieties of wine, such as Sauvignon blanc and Pinot noir. The predictor set is limited to very basic information, including the winemaker's geographical location, the current portfolio of wine varieties other than the one being modeled, and the change in that portfolio over time. The primary data set is the Wines & Vines 2000 Varietal Chart, produced by The Haring Company of San Rafael CA (*Wine Varietals Made by U.S. Winemakers: Vinifera*, Wines & Vines, December 2000, pp. 30-41). The methodology employed is a non-parametric, non-linear classification tree structure that has shown great promise relative to neural networks and other data mining tools. The resulting analysis produces a decision tree that any winemaker can easily traverse to see whether current *vinifera* grape production decisions are consistent with other statistically similar winemakers, thereby identifying varietal markets to consider for expansion or contraction.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Information Management Tools for Agriculture, #4: Spatial Distribution of Mycobutanil in Chardonnay Grape Vineyards and Wine**

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The California Department of Food and Agriculture GC/MS Pesticide Scan method is a useful technique to examine the distribution of pesticides on grape bunches in a vineyard. We have shown that maps from processed aerial image NDVI give zones and resultant samples of varying levels of mycobutanil in Chardonnay grapes grown in Monterey, CA. Stuck fermenting barrels of Chardonnay grape juice showed higher levels of mycobutanil than barrels with normal fermentation. Filtration of the wine did not remove the mycobutanil. No mycobutanil was detected in the final wine.

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# Role of Different Oak Wood Chips in Releasing Non-Volatile Phenolic Compounds into Model Solutions

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Several procedures have been employed to impart a woody character to the wine without utilizing expensive barrels; for example, the soaking of oak wood chips. Current studies exist concerning the flavouring capability of these chips. Besides adding flavours to wine, an important function of barrels is to stabilize colour and to enhance the phenolic structure in wines. In this work, the role of different oak wood chips in releasing non-volatile phenolic compounds has been investigated. Chips produced from French and American oak at three different toasting levels and sizes were immersed in solutions simulating wine. The release of non-volatile phenolic compounds has been studied measuring the absorbance from visible wavelength to the ultraviolet ones at different times.

We found that the use of chips only as flavouring substances is quite limitative. Lightly toasted chips release polyphenols at higher concentration compared to those more toasted. The release of phenolic compounds is quickest using small chips and because of that they could be suitable to obtain a rapid stabilization of wine colour during fermentation. Larger chips and barrels have a similar effect on wine. Release of phenolic compounds was studied by measuring the absorbance of similwine to which French and American oak chips were added separately. French oak chips add more phenolics than their American counterparts. The higher the pH and the alcohol content of the similwine solutions, the greater is the extraction of phenolic compounds. The American oak (*Quercus alba*) is denser than the French oak (e.g., *Quercus petraea* or *Quercus robur*). We believe that the different density of the two woods influences the rate of transfer of chemical compounds from chips to similwine. We may conclude that the winemaker has at his/her disposal powerful and flexible tools considering the availability of chip sizes, toasting levels, wood species and the choice of timing of application. According to the winemaker's objectives, chips can be used either as flavouring agents or as stabilizing agents to improve the quality of colour of wines.

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# Role of Different Oak Wood Chips in Releasing Volatile Compounds into Solutions Simulating Wine

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Several procedures have been employed to impart a woody character to the wine without utilizing expensive barrels. The aim of this research has been to study how different levels of toasting, size and origin (American and French) of wood chips can affect types and quantities of volatile compounds released in wine. Chips produced from French and American oak at three different toasting levels and sizes were immersed in solutions simulating wine. The release of volatiles has been studied. Techniques used for the aroma analysis were: GC-MS, HRGC and Gaschromatography-Olfactometry. The following compounds were identified in the solutions: *trans* and *cis* b-methyl-g-octolactone, phenolic aldehydes, volatile phenols, terpenes, norisoprenoids, and furanoic compounds.

Toasted chips were found to be flavoring agents: the quantity of aromatic compounds increases with the toasting level and alters the type of compounds in relation to the "light" chips. Some aromatic components are lost by volatilization as a result of intense thermal treatment while the quantity of others increases. The maximum concentration of volatiles is found in medium toasted chips. Generally the medium and heavy toasting levels differ in relation to the absolute and relative concentration of the aromas, hence the organoleptic sensation resulting from their usage can be different. The finer the size, the easier is the migration of volatile compounds into solution. However, some compounds are "lost" in the oak wood milling process. French and American oak have a different aromatic composition. The potential of oak chips seems to be vast, especially if we consider to possibility of aggregating different types to obtain mixtures. It should be also noted that chips yield non-volatile phenolic compounds. The winemaker must be careful in choosing the right product and appropriate timing of application to affect the aroma only.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

## ***Vitis vinifera* L. cv. Tannat Produces the Typical Red Wine of Uruguay**

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An overview of the amount of *Vitis vinifera* planted in Uruguay shows that Tannat grapes represent 32.2% of the vineyard. Although it originated in the South of France, today it is almost unknown in Europe. This French red variety acclimatized very well to the cool and humid conditions of Uruguay since 1870, and today high quality wines are starting to be produced. Initial research has been carried out to characterize the complex flavor described as dried vegetative, smoked and empyreumatic scents, prune and quince notes, blackcurrant and other small wood fruits-like descriptors, particularly through possible peculiarities in the free forms and possibly also in the heteroside fraction. In this research on the aroma components of wines of the Tannat variety, we focused attention principally on compounds possibly linked to varietal peculiarities, extending in particular the investigation to other monoterpenols and to the bound forms, with the aim to improve the characterization and classification of the wines. Volatiles and precursors have been quantified after adsorption and separate elution as previously reported. HRGC-FID and HRGC-MS or SIM/MS instrumental procedures using an internal standard (1-heptanol) were applied for identification proposals as previously described. The most noticeable compounds were the aglycones obtained after enzymatic reaction. In particular, we mention the presence of some monoterpenols like furan and pyran linalool oxides,  $\alpha$ -terpineol, nerol, geraniol and above all the *trans* form of the 8-hydroxylinalool. A remarkable presence of norisoprenoids as 3-oxo- $\alpha$ -ionol, 3-oxo-7,8-dihydro- $\alpha$ -ionol, 3-hydroxy- $\beta$ -damascone, 3-hydroxy- $\beta$ -ionone, 4-oxo- $\beta$ -ionol and 4-oxo-7,8-dihydro- $\beta$ -ionol has also been found when comparing the MS spectra with those of the recent literature.

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# **The Phenolic Quality of Red Grapes: A New Objective Evaluation System by Colour Measurement**

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The current research evaluated the possibility of the real time (before pressing) measurement of the phenolic quantity of red grapes to accurately define the true quality of the raw material destined for the production of wine. The measurement procedure involved the use of a spectrometer and the acquisition of a spectral signal via fibre immersed directly in the sample to be analyzed. The measurements were performed on the same juice used for refractometry analysis of the sugars, and it was established that it was possible to objectively classify the red grape cultivars on the basis of their anthocyanin and total polyphenolic content. The colour of red grape juice at delivery, for all the maceration techniques, was correlated to the colour intensity of wine. The parameter "colour" can be acquired instantly and the data used in real time to improve the management of wine-making process and also pay for the grapes on the basis of their phenolic quality.

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# Evaluation of Wine Haze and Aggregate Formation Using a Model Wine System

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A one-half fractional factorial statistical design was used to evaluate six factors important to haze and aggregate formation in a model wine system. The six factors chosen to vary at high and low levels were protein, polyphenols, sucrose, pectin, acid, and ionic content. The model wines were evaluated using size exclusion chromatography, laser light scattering and spectrophotometry. Evaluations were made on the molecular weight, root mean square radius, peak height, peak range, and protein content by using size exclusion chromatography and laser light scattering. The haze potential of the model wines was determined using a heat test and spectrophotometry. By evaluating the statistical results, a determination was made that the interaction of sucrose and pectin was significant for the molecular weight of the particles in the model wines. The root mean square radius values of the model wines were found to be significant for the interaction of polyphenols, sucrose, and pectin. Peak height results were found to be statistically important for the interaction of polyphenols and acid and also for the interaction of polyphenols, sucrose, and acid. Peak range results were statistically significant for the main effect of protein. The protein content for the model wines was also significant for the main effects of protein, polyphenols and for the interactions of protein and polyphenols, and also for the interaction of protein, polyphenols and acid. The haze potential results showed the greatest significance for the main effect of polyphenols.

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# Alcohol in Wines by Near Infrared Detection

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This study uses a near infrared (NIR) spectrophotometer with autosampler to determine the alcohol content in red, white and rose standard wine. The NIR instrument employed measures the absorbance in a highly alcohol-specific spectral range (between 1150nm and 1200nm) and uses a powerful evaluation algorithm to determine the alcohol content. The influence of other sample components is essentially eliminated. Sample preparation is minimal and components is essentially eliminated. Sample preparation is minimal and involves degassing carbonated samples and filtering turbid samples. The sample run time is about 2 minutes per reading. Calibration of the instrument requires only a two point traceable standard water and a known water/ethanol standard. The usual method for screening wine samples for alcohol content in this laboratory is gas chromatography (GC/FID) with internal standard. Over 200 samples (run in triplicate) comparing the NIR and GC data were analyzed over a three week period. Results for both the NIR and GC/FID are presented.

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# Evaluation of the FOSS WineScan FT 120 for Rapid Automated Routine Quality Control Wine Analyses

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The WineScan FT 120 Fourier Transform Infrared (FTIR) analyzer from FOSS was found capable of automatically analyzing for 8 wine constituents simultaneously in 30 seconds. The combination of FTIR, chemometrics and compositional data of wines obtained from reference methods were used to establish calibrations for ethanol, total acid, pH, volatile acidity, reducing sugars, tartaric, malic and lactic acids. To date a total of 263 wines in 6 different wine categories were used for the WineScan calibrations. The wine categories were dry white, sweet white, blush, red, dry red premium and flavored wines. Good correlation was obtained with the reference laboratory methods for all constituents except the organic acids. To fully optimize the organic acid correlations, more calibration samples will have to be input. Advantages of the WineScan are speed, automation (64 or 120 sample tray), no sample pretreatment except degassing and possible filtration, low maintenance cost, significant chemical and labor savings. The WineScan can be a significant asset to the quality control laboratory if the type and number of analyses warrant it.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Evolution of Anthocyanin-Derived Pigments in Ageing Red Wines

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The colour changes during wine maturation are usually attributed to the formation of new pigments resulting from the interaction between anthocyanins and other phenolic compounds, especially flavan-3-ols like catechins and procyanidins (condensed tannins). Nevertheless, new anthocyanin-derived pigments have been reported in red wines arising from the reaction of grape anthocyanins with other small molecules like pyruvic acid. These newly formed pigments were shown to be more stable and stabilise wine colour changing it to a more brick red hue. All these anthocyanin-derived pigments were previously extracted from Port wines and structurally identified by NMR techniques and mass spectrometry. They were referred to as malvidin 3-glucoside-pyruvic acid derivative (mv-py) and its respective acetyl (mv-ac-py) and coumaroyl esters (mv-coum-py). Calibration curves were performed using malvidin 3-glucoside (mv) and mv-py as standards. The evolution of these pigments was followed in several Port wines along with the other original grape anthocyanins, regarding their stability and contribution to wine colour, during 38 months. Amongst all pigments analysed (including anthocyanidins monoglucoside), mv-py was found to be the most stable. After one year of oak ageing, the original grape anthocyanins decreased in amount by 75 to 90% whereas mv-py decreased by only 15 to 25%. At this stage, most of the wine colour is due to polymerised pigments. After three years of oak ageing, malvidin 3-glucoside, the major original anthocyanin, was not detected in all wines studied whereas its pyruvic acid derivative was still detected. Concerning the relevance of these newly formed pigments to wine colour, their hypsochromic spectral shifts from mv indicate a shift to orange-brown at wine pH.

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# Misidentification of Odours: Perceptual Bias and Semantic Memory in Expert and Novice Wine Judges

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Recent evidence argues that much of the taste and smell of a wine is “not in the bottle, but is in our mind” (Brochet, 1999). The present study investigated cognitive errors associated with olfactory-guided judgments as a function of domain-specific expertise. Despite similar olfactory sensitivities, expert and novice wine judges were predicted to differ, qualitatively and quantitatively, in terms of their erroneous judgments of wine-relevant, olfactory stimuli. Expertise is defined in terms of a wine judge’s verbal (semantic) and olfactory experience with wine-relevant stimuli (Melcher & Schooler, 1996). Twenty odorants were sampled ortho-nasally in odour-identification and odour-recognition tasks. In a subsequent discrimination task, experts and novices discriminated experimenter-specified odours in four model wines. Two of the four wines were identical in odour but differed on the basis of colour, where the colour manipulation simulated typical age-related change or oenological-processing change. Expert wine judges were predicted to have a higher probability of making judgment errors based on perceptual bias than novices (Cain & Potts, 1996), while novice judges were expected to have greater difficulty finding verbal labels for the odorants. Results are interpreted within an associative memory framework where previously learned odour associations are predicted to influence judgment behaviour. In terms of meta-cognition, novice judges were expected to be aware when their odour-guided judgments were erroneous whereas experts may be particularly confident that their erroneous judgments are correct, suggesting that vulnerability to errors of perceptual bias is a more insidious and potentially serious problem in wine judgments.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Compositional Differences of Charred and Non-charred French and American Oak and French Reeds Used in Cooperage

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There have been numerous studies of the chemical composition of oak cooperage wood, particularly of the ellagitannins and of volatile compounds such as lactones and volatile phenols. In contrast, there is little data concerning the levels of saturated and unsaturated fatty acids or of carbohydrates in oak cooperage. Furthermore, there is no available information on the chemical composition of reeds (*Juncus*). Dried reeds are used in coopering as "flagging" to ensure that barrel headpieces do not leak. A strip of reed is placed between each section of the headpiece, which is then assembled using dowel pins. The aim of this study was to compare the composition of reeds with French and American oaks. The pH, carbohydrate, fatty acid and volatile composition were measured for several charred and non-charred samples of French (*Quercus petraea*, *Quercus robur*) and American oak (*Quercus alba*) of known origin, selected by the cooperage company Tonnellerie Radoux (Jonzac, France). Glucose and xylose were the major carbohydrates in all the samples, while ribose was the least prevalent. The carbohydrate composition of reeds was similar to that of oak wood, but the overall content was higher. The major fatty acid in non-charred wood was C:24 and then C:26, while C:16 was the major fatty acid in reeds. In general, charring decreased the overall fatty acid content. Certain volatile compounds after the charring of wood and reeds were the same. A new derivative of maltol was identified after the heating of oak wood. There was no statistically significant difference in the pH between French and American oaks. Charring resulted in an alkaline pH (8.62) for reeds and an acid pH (3.60) for oak wood. In general, pH increased with charring. Overall, reeds composition was different than the woods.

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# **Influences of the Different Forms of Anthocyanins (Copolymerized, Free, and Polymerized) on Color in a Young Red Wine**

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Anthocyanins are responsible for the red color in red wines. Intermolecular copigmented anthocyanins, anthocyanin polymerization, and anthocyanin monomers have been examined to evaluate their effect on color intensity and stability during the first year of wine aging. Initial levels of intermolecular copigmented anthocyanins seem to play a role in increasing color intensity and stability. However, copigmentation's role in color intensity appears to decrease consistently from the end of fermentation through the aging of the wine. Pinot noir from the Edna Valley and Cabernet Sauvignon from the Napa Valley were spectrophotometrically analyzed postfermentation through eight months of aging to determine the influence of each form of anthocyanin on the red color. Preliminary conclusions tend to suggest that intermolecular copigmented anthocyanins tend to play a more significant role in red wine color in the Cabernet Sauvignon than the Pinot noir.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Grape Spirit Aroma Contribution to the Complexity of a Single Varietal Port Wine

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Port wine is a complex aromatic beverage composed of four major groups of volatiles: primary volatiles characteristic of the grape; secondary or yeast fermented volatiles; tertiary volatiles formed/extracted during bottle or cask ageing; distillation volatiles, an important and previously unconsidered component from the fortification spirit (aguardente). The present study investigates the important contribution made from the aguardente relative to the fermented wine fraction. Four single varietal Tinto Cão Port wines from the 2000 vintage, were made from the same batch of fermenting juice (initial °Baumé: 12.95), being fortified with either aguardente (77% v/v EtOH) or aqueous ethanol (77% EtOH) at two different apparent °Baumé values: 9.50 and 8.25. The Ports and the aguardente were quantitatively examined for a total of 26 volatiles by either GC-FID or GC-MS: 10 esters, 3 alcohols, 2 terpene alcohols, 1 phenol, 1 aldehyde, 1 acetal, and 8 acids. Predominant compounds originating from the aguardente included: ethylbutanoate (>99%); ethylhexanoate (>73%); ethyllactate (>77%); cis-3-hexenol (>92%); ethyloctanoate (>68%); benzaldehyde (>76%); linalool (97%); ethyldecanoate (>69%); diethylsuccinate (>60%); *a*-terpineol (>98%); eugenol (>61%); pentanoic acid (>99%); octanoic acid (>75%); decanoic acid (>78%). Compounds more evenly distributed included isoamylacetate, 2-phenylethylacetate, ethyldodecanoate, ethylhydrocinnamate, and hexanoic acid. Benzyl alcohol (>63%) and 2-phenylethanol (>78%) were principally components from the fermented wine. The aguardente acetal, 1,1,3-triethoxypropane, rapidly hydrolysed following juice fortification, resulting in zero wine levels. Aroma profiles were obtained by olfactory gas chromatography (GC-O) of diethylether/hexane volatile extracts of both the aguardente and fortified wines. Sensory evaluation indicated the aguardente fortified wine to be exceptionally more complex than the fortified ethanol control, having particularly more intense notes of exotic fruit, floral, resin, and spice. Fortification with aguardente increased numerous component concentrations to around or above known threshold levels (Odor Unit > 1), emphasizing that wine spirit is an essential contributor to the aroma complexity of Port.

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# Quantitative Analysis of Volatiles Present in Wine Spirits Used for Port Production

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Wine spirit or "aguardente" as it is commonly known in Portugal, is used for the fortification of fermenting grape juice for the production of Port, and is an important source of flavour compounds. The present study quantified 26 volatile compounds present in 7 aguardentes of Portuguese, Spanish and French origin. The aguardentes were diluted, 1 in 5 volumes, with synthetic fermenting must (6.25g/L tartaric acid; 5.7% EtOH; pH 3.25) in order to give final alcoholic strength 20% v/v and pH 3.5, corresponding to typical Port levels. Quantification gave the following concentration ranges found in the 7 diluted aguardentes: Esters: isoamylacetate (367-796mg/L); ethylbutanoate (217-773mg/L); ethylhexanoate (203-652mg/L); ethyllactate (16.0-67.1mg/L); ethyloctanoate (1.38-3.14mg/L); ethyldecanoate (1.12-4.80mg/L); diethylsuccinate (1.12-2.93mg/L); 2-phenylethylacetate (20-98mg/L); ethyldodecanoate (144-1074mg/L); ethylhydrocinnamate (1.1-3.4mg/L). Acids: acetic (Not Detected (N/D)-84mg/L); isobutyric (N/D-759mg/L); isovaleric (N/D-1112mg/L); pentanoic (N/D-610mg/L); hexanoic (trace-1036mg/L); octanoic (91-3325mg/L); dodecanoic (N/D-233mg/L). Alcohols: benzyl alcohol (18-92mg/L); 2-phenylethanol (1.09-3.60mg/L); cis-3-hexenol (266-1577mg/L). Terpene alcohols: linalool (8-100mg/L);  $\alpha$ -terpineol (4.5-43 mg/L). Acetal: 1,1,3-triethoxypropane (147-507mg/L). Aldehyde: benzaldehyde (56-127mg/L) Phenol: eugenol (3.4-4.9mg/L). The volatile contribution made from the wine spirit fraction to Port for many of these compounds is already above the known literature sensory threshold levels, giving odor units (OU) >1. Examples include ethylbutanoate, ethylhexanoate, ethyloctanoate, ethyldecanoate, ethylhydrocinnamate, octanoic acid and linalool. This emphasizes the important odor contribution made by wine spirit volatiles to the complexity of Port. Considerable differences were observed between the aguardentes of Portuguese and Spanish origin compared to those from France. The latter contained considerably less esters (fruity odors) and very little organic acids (sweaty/cheesy odors). Knowledge of the aroma profile for different commercial wine spirits will give the winemaker a better understanding, aiding the selection of a particular aguardente for a particular wine style.

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# HPLC Analysis of Prolyl Endopeptidase Inhibitors in Wine Related to Beneficial Effects on Memory

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Prolyl endopeptidase (PEP) has been reported to be abundant in the hippocampal region of human brain in amnesic patients. High levels of PEP hydrolyze neuropeptides involved in neurotransmission, memories, etc., and the memory function is deteriorated. The PEP inhibitors are expected to be the remedy for memory dysfunction. As there is no report on the PEP in wine, we examined the occurrence in red wine produced from Cabernet Sauvignon grapes harvested in our experimental vineyard, Johnohira, Katsunuma, Japan. Two PEP inhibitors were isolated from the wine and determined the structures as Val-Glu-Ile-Pro-Glu (A) and Tyr-Pro-Ile-Pro-Phe (B). The peptides inhibited degradation of humane neuropeptides such as vasopressin, substance P, and neurotensin by PEP. We examined the content of the PEP inhibitor (A) in commercial wines. The peptide inhibitors were partially purified with an Oasis HLB cartridge and the contents were analyzed by HPLC with a CAPCELLPAC C18 column. The peptide content was confirmed by the ELISA assay using an antibody to the peptide A. The peptide A contents were 230, 257, 218, and 520 mg/L in Cabernet Sauvignon wine produced in Chile, USA, France, and Bulgaria, respectively. The peptide A contents were 100, 416, 252, and 530 mg/L in Merlot wine produced in Chile, USA, France, and Bulgaria, respectively. Chardonnay wine also contained the PEP inhibitors and the peptide A contents were 80, 194, 157, and 146 mg/L in the wine produced in Chile, USA, France, and Bulgaria, respectively. As red and white wines contain PEP inhibitors, wine ingestion in moderation may be beneficial for inhibition of the deterioration of memory function.

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# Evidence for Yeast Flora Selection by the Winery Environment

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Yeasts associated with the man-made ecological niche of the winery play an important role in both spontaneous and inoculated wine fermentations. Several studies have shown that the winery environment is colonized by many cells of *Saccharomyces cerevisiae* which go through innumerable generations at each vintage. There is probably a significant selective pressure by conventional limiting factors such as ethanol and SO<sub>2</sub> on the *S. cerevisiae* population of the winery. In order to investigate the influence of the winery environment on enological characters of the resident *S. cerevisiae* population we carried out an ecological study in two different wineries in the Umbria region (Italy). One of these is a modern, working winery (about 8,000,000 L of must/vintage), the other has not been operating since 1914 and is located in an artificial cavern characterized by having constant temperature and humidity. Using enrichment procedures, we isolated and classified the yeast flora from different surfaces of both wineries. The *Saccharomyces* populations coming from industrial winery (40 strains) and the former cavern winery (23 strains) were evaluated for several physiological and technological characters. Results showed that flocculent and H<sub>2</sub>S<sup>-</sup> strains were present in the industrial winery. In addition many strains showing SO<sub>2</sub> resistance and killer activity were found. On the contrary, strains isolated in the former winery showed only limited diffusion of killer activity and SO<sub>2</sub> resistance. Statistical differences were found between the two *Saccharomyces* populations regarding such analytical characters as: maximum ethanol production, fermentation rate, malic acid degradation, acetic acid production. In particular, characters linked to selective pressure such as maximum ethanol production and fermentation rate were higher in the strain population coming from the industrial winery than those isolated from the other. These results demonstrate that the winery is a selective environment and that the presence of an enologically competent microflora is associated with the continual selective pressure of a working winery with successive vintages.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Rapid Detection of Wine Lactic Acid Bacteria by DEFT Application to Microbiological Control of Wine and Contact Surfaces**

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The detection of populations of lactic acid bacteria at different stages of the wine production process plays an important role in quality control especially in the cases of sweet and sweet-fortified wines. The detection of these organisms by traditional cultural methods is not always the most appropriate solution. One reason for this, aside from the obvious slowness, being the difficulty encountered in growing some of these organisms directly from the wine environment. The direct epifluorescent filter technique (DEFT) is an alternative in these cases. In this work a bacterial viability procedure, previously studied in our laboratory, which utilizes a mixture of two fluorescent nucleic acid stains (SYTO 9 which labels all bacteria in a population, and propidium iodide which penetrates only bacteria with damaged membranes - competing with the former for nucleic acid binding sites), was used for the detection of wine lactobacilli. The method was applied to wineries, bottling lines, cellars and wine transportation tanks. Contaminant bacteria were mainly found in presses and pumps (wineries); filler heads (bottling lines); pipe lines (cellars) and tanks and taps (wine transportation tanks). For practical purposes the rapid method used seems to be a good alternative to the traditional cultural methods as part of quality control programmes in winemaking.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Survival of *Hanseniaspora guilliermondii* at High Concentrations of Ethanol

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The early stages of the alcoholic fermentation are characterized by the activity of mostly apiculate non-*Saccharomyces* yeasts, these strains dying off as fermentation progresses leaving *S. cerevisiae* to predominate. It is therefore, generally accepted that non-*Saccharomyces*, fermenting yeasts have little impact on the final quality of most wine styles. In this study the survival of a strain of *Hanseniaspora guilliermondii* was monitored during mini-vinification of red grapes (Tinta Roriz from the Dão region of central Portugal). Two fermentations (100 L each from the same batch of grapes – one inoculated with *H. guilliermondii*, one spontaneous) were carried out using the traditional foot treading technique. For the experimental fermentation a fresh inoculum of *H. guilliermondii* (1% v/v) grown in aerated conditions was added immediately after crushing giving an initial concentration of about  $10^7$  cfu/mL. Samples were taken along the fermentation – yeasts being counted on YM agar (Difco) plates, identification of typical colonies being made using standard methods and with reference to the scheme of Barnett *et al.* (1994). Total sugars (glucose and fructose) and ethanol content were analyzed by HPLC. As expected, after 4 days of fermentation (4% v/v ethanol), *S. cerevisiae* became the dominant species in both fermentations, although *H. guilliermondii* was present in countable numbers almost until the end of the inoculated fermentation ( $10^3$  cells/mL at 12.5% v/v ethanol). Another apiculate yeast, identified as *H. uvarum*, was found to remain until the last days in both fermentations. These results indicate that certain strains of apiculate yeasts are able to survive to higher ethanol concentrations than 4-6% (v/v) most often described in the literature. Further experiments on the kinetics of inactivation at high concentrations of ethanol have been showing that *H. guilliermondii* behaves in a similar manner as *S. cerevisiae*.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Characterisation of Lactic Acid Bacteria Strains from Rioja Red Wines and Responsible for Malolactic Fermentations

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The objective of this work was to characterise lactic acid bacteria which constitute the indigenous microflora of red wines of the Spanish northern region of Rioja, and which are as well responsible for malolactic fermentations. Traditional malolactic fermentations are carried out spontaneously after alcoholic fermentation and are led by endogenous lactic acid bacteria, which maintain in latency during yeast fermentation. Screening was performed in a total of 20 wineries representative of the three Rioja subregions and samples were collected from 1994 to 1999 during traditional vinifications of red wines (cv. Tempranillo, Garnacha, Mazuelo and Graciano). Samples were grown in MRS-agar at 30°C, 98% humidity, and either under 10% CO<sub>2</sub> atmosphere or total anaerobiosis. Identifications were performed by morphological and biochemical tests and by PCR. Specific primers for *Lactobacillus plantarum* (Quere et al. 1997) and *Oenococcus oeni* (Dellaglio et al. 1998) were used. Strain characterisation was carried out by Pulsed Field Gel Electrophoresis utilizing *Sfi*I restriction enzyme and BioRad CHEF-DR II system. Two major species were found: *Lactobacillus plantarum* and *Oenococcus oeni*, which constituted 89% of total isolates. Regarding strain diversity, a wide variety of restriction patterns corresponding to clones of the same species were found. More than one strain was found simultaneously in the same fermentation deposit. These results showed the wide biodiversity of lactic acid bacteria in Rioja wines. Ten *O. oeni* clones and one *L. plantarum* clone were shown to be predominant in more than one winery. These strains have, therefore, optimal characteristics for growing in Rioja wines and leading malolactic fermentations.

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# **A Survey of Yeast Microflora in Barrel Fermented Chardonnay Fermentations Using Direct Molecular Methods**

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We have recently developed molecular techniques (PCR-DGGE) to identify microorganisms directly from wine fermentations. With these methods, we are able to characterize the yeast microflora from a very small volume of juice, often as little as 100 microliters. Moreover, samples that have been frozen for six months or more still give accurate microbial profiles using PCR-DGGE. During the 2000 harvest, we collected nearly 1,000 wine samples from over 50 barrels of Chardonnay produced in the Napa Valley. Our goals were as follows: (1) to use PCR-DGGE to survey the normal yeast microflora in barrel fermented Chardonnay, (2) to examine the microbial variation within one winery from barrel to barrel, (3) to examine the microbial variation between three different wineries, and (4) to study the effects of inoculated versus uninoculated fermentations within one winery and between wineries. A comprehensive understanding of the "normal" yeast microflora present in these fermentations will aid in predicting stuck fermentations in the future.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Yeast and Bacteria Interactions in Chardonnay from Northern California

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Production of wine has been conducted for several hundred years by spontaneous alcohol fermentation of grape juice. *Saccharomyces cerevisiae* is predominant in fermentation of wine due to its adaptation to extreme conditions prevailing in the grape must, like low pH (2.8-3.6) and high glucose/fructose concentrations (150-280 g/L) and during fermentation, high ethanol concentration (up to 18 vol%). The second fermentation in wine is the so-called malolactic fermentation (decarboxylation of malate to lactate), which is conducted predominantly by *Oenococcus oeni*. This decarboxylation stabilizes the wine microbially, increases the pH of the wine and decreases the total acidity, giving a more pleasant mouthfeel when drinking the wine. It is still difficult, and in several cases impossible to induce malolactic fermentation in some types of wines, especially white wines, and Chardonnay in particular. This has been attributed to the antagonistic interaction between yeast and bacteria, where *O. oeni* are nutritionally fastidious and have complex nutrient requirements. *S. cerevisiae* may deplete the wine for nutrients required for proper growth of the malolactic bacteria. This is more profound in white wines due to the absence of or short grape skin contact. In addition, the yeast produces inhibiting end-fermentation products such as ethanol and SO<sub>2</sub>. The effect of these antagonistic interactions with *O. oeni* is greatly influenced by the choice of yeast starter culture. A study of the alcohol fermentation in two different Chardonnay grape juices fermented in barrels by the yeast EC 1118 and a new Chr. Hansen wine yeast, respectively, have been conducted. The trials were conducted at two wineries in Napa Valley during the vintage 2000. Furthermore, the very different antagonistic effect by the two different yeast strains on the concomitant malolactic fermentation, conducted by several commercial available bacteria starter cultures, will be presented and discussed. These findings clearly show the necessity to select a proper yeast starter culture by the winemaker, in order to secure a fast and reliable malolactic fermentation.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Detection of Genes Related with Bacteriocin Production in Endogenous Lactic Acid Bacteria from Rioja Red Wines

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During malolactic fermentation, there is a competition among lactic acid bacteria, and the synthesis of bacteriocins by some of these microorganisms could be a factor of influence in the ecology of wine. The objective of this work was to study the regulatory operon *pln*ABCD as well as other genes related with bacteriocin production (*pln*K, *pln*N and *pln*EF) in lactic acid bacteria strains from Rioja red wines. Lactic acid bacteria strains (39) were studied, which included the following species: *Lactobacillus plantarum*, 21; *Lb. brevis*, 6; *Leuconostoc mesenteroides*, 6; *Lb. paracasei*, 2; *Pediococcus pentosaceus*, 1; *P. damnosus*, 1; *P. acidilactici*, 1; and *Lactococcus lactis lactis*, 1. Nine out of the 21 studied *Lb. plantarum* strains showed bacteriocin activity (Bac-positive) and the other lactic acid bacteria strains showed no antimicrobial activity (Bac-negative). Primer sequences for PCR analysis were chosen with help of GCG (Genetics Computer Group) software package. All Bac-positive *Lb. plantarum* isolates showed the *pln*ABCD operon genes, that is: plantaricin A precursor *pln*A, and the three regulatory genes: B, C and D. Six Bac-negative *Lb. plantarum* isolates also showed the *pln*ABCD operon genes, and in one Bac-negative *Lb. plantarum* isolate, only the *pln*A and *pln*C genes were detected. None of the other 23 Bac-negative lactic acid bacteria isolates (including 5 *Lb. plantarum*) showed the *pln*ABCD operon genes. *Pln*K, *pln*N and *pln*EF genes were detected in 13 *Lb. plantarum* isolates (6 Bac-positive and 7 Bac-negative) in which the *pln*ABCD operon genes were previously shown. In conclusion, a variety of genes related with bacteriocin production were detected in *Lb. plantarum* isolates of Rioja red wine and this fact is of interest to understand the ecology of lactic acid bacteria during malolactic fermentation.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Micro-oxygenation Influence on the Tannin, Color, Clarity and Aroma Characteristics of Three Types of Merlot Wines

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Micro-oxygenation (MOX) was evaluated in Merlot wines expressing vegetal, fruity, and tannic characteristics. The MOX procedure infuses oxygen into post-fermentation wine at a minute rate (ml O<sub>2</sub>/L/Month) preventing dissolved oxygen accumulation. This type of addition triggers an oxidative coupling reaction resulting in phenolic modification, i.e., anthocyanin and tannin condensation by way of acetaldehyde. Tannin evolution, color stability, clarification, and aroma development were monitored qualitatively and quantitatively over time. Each wine type had different phenolic profiles with each profile determining the amount of oxygen to add. Preliminary qualitative results showed improved mouthfeel and fruit intensity in all MOX treated wines. In consecutive organoleptic evaluations, the fruity wine type expressed intensified ripe fruit flavor and aroma. Tannins varied from characteristically sharp, closed, and young to those described as 'round' and 'melted.' Clarification differed among the wine types. The fruity wine type clarified after one week of MOX treatment at 65 °F while the tannic and vegetal wine types proved difficult to clarify. Adding oxygen to the vegetal wine did not improve its aroma and flavor though it did slightly soften the tannins. MOX has the potential to produce desirable changes in wine texture and aroma that cannot be obtained by traditional aging techniques. However, further research is required to determine the best wines for MOX treatment and the appropriate dosing level to improve wine quality.

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# Determination of Carotenoid Profiles in Grapes, Musts and Fortified Wines from Douro Varieties of *Vitis vinifera*. Role of Alcoholic Fermentation on Carotenoid Levels In Fortified Wines

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The most abundant carotenoids present in red grape varieties are  $\beta$ -carotene, lutein, violaxanthin, luteoxanthin and neoxanthin. These large non-aromatic molecules are known to be precursors of aroma-active substances responsible for the typical aroma of some grape varieties - their biological and chemical degradation leading to the formation of certain C13 nor-isoprenoid compounds such as  $\alpha$  and  $\beta$ -ionone or  $\beta$ -damascenone, compounds associated with positive sensory attributes in wines. In order to study the profiles of carotenoids in grapes, musts and fortified wines from red Douro varieties of *Vitis vinifera*, an HPLC method allows the determination of  $\beta$ -carotene, lutein and other xanthophylls (neoxanthin, violaxanthin and luteoxanthin). Despite the fact that the carotenoid levels found in fortified wines (768 $\mu$ g/l) were lower than those found in musts (1691  $\mu$ g/L) their presence in wines suggests a possible direct conversion into nor-isoprenoids as b-damascenone. In this work the carotenoid profile was followed during fermentation. Levels of lutein and  $\beta$ -carotene decrease drastically in the 2 first days 510  $\mu$ g/L to 125  $\mu$ g/L for lutein and 980  $\mu$ g/L to 215  $\mu$ g/l for  $\beta$ -carotene. However neoxanthin, violaxanthin and luteoxanthin that are non-existent in musts and increase in the 3 first days of fermentation. After this their levels decrease and in sec red wine they were not found. Because in a fortified wine fermentation stops when sugar concentration is 200-250 g/L final wines have important levels of neoxanthin, violaxanthin, luteoxanthin (which can attain 214  $\mu$ g/L).

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Balkan Oak: An Economic Alternative Source for Quercus

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Chardonnay from two vineyards in northern California was barrel fermented and aged in Balkan oak (*Quercus petraea*) that had been seasoned for 6, 12, 15 and 18 months then built into medium toast 225 litre barrels (World Cooperage Co Inc.). The wines were submitted to chemical and sensory analysis and preference tested against wine from comparable French and American oak barrels. The chemistry of the oak was monitored during seasoning and after toasting. Early indications suggest that it may reach its optimum age earlier than French oak. Chemical and sensory evaluation of both wood and wine showed that Balkan oak, while clearly European in character had some American oak characteristics notably in its 'toasty' character. In one chardonnay Balkan oak gave the most spicy and smoky wine while in the other it showed a lot of tannin which may marry well with time. The ultra-structure of the oak was similar to French oak and typical of European cooperage oak. However the early wood vessels were well occluded with tyloses and in many of the oak samples the parenchyma rays were empty although tannin levels were similar to French oak. The average grain tightness tended to be finer than French and American oak of comparable quality. Chemical analyses of the seasoning oak suggest it may not require as long a period as French oak. In preference tests by a group of 44 winemakers 25% preferred the wine from Balkan oak.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Effects of Powdery Mildew Infection Rates on Single Leaf Photosynthetic Efficiency on Potted Grapevines

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Potted, one-year-old, own-rooted 'Chardonnay' grapevines (*Vitis vinifera* L., Colmar clone) were grown in a greenhouse, and either: 1) inoculated with a conidial suspension of powdery mildew (*Uncinula necator* (Schw.) Burr.), or 2) not inoculated. Powdery mildew was additionally controlled on the noninoculated vines by spraying with NOVA fungicide (Myclobutanil) as deemed necessary based on visual assessments of disease incidence. Leaf photosynthesis measurements were conducted on the most recent fully expanded leaves on each plant at three-week intervals during the growing season using a portable infrared gas analyzer fitted with a leaf cuvette. Powdery mildew infection was determined visually and expressed as a percentage of total leaf area. Net carbon assimilation was correlated with disease incidence on individual fully expanded leaves during the growing season, and on total leaf area at the end of the season. Damage thresholds were determined by estimating the minimum degree of infection necessary to result in decreased single leaf Pn efficiency and reduction in total vine leaf area.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Genetic Control of Phylloxera Resistance

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Grape phylloxera, *Daktulosphaira vitifoliae* (FITCH), is the most important insect pest of grape and continues to impact the world's vineyards. It is native to North America, but has spread to every region where grapes are grown, causing billions of dollars in damage. Rootstocks, bred from resistant North American grape species, have been used to control phylloxera for over 100 years. However, rootstocks with partial *Vitis vinifera* parentage (used for its lime tolerance and good propagation) fail to phylloxera, and phylloxera strains appear to be adapting to rootstocks with moderate levels of resistance. This project is examining the phylloxera resistance of *V. rupestris* and the susceptibility of *V. vinifera* by examining progeny from a remake of AXR#1 (*V. vinifera* 'Aramon' X *V. rupestris* 'Ganzin'). Seedlings from this F1 progeny were selected, and a series of crosses among resistant and susceptible individuals and backcrosses to 'Aramon' and 'Ganzin' were made. One hundred and fifty seedlings, selected from these crosses, were infested with phylloxera to identify resistant and susceptible plants and to determine inheritance of the resistance and susceptibility traits. Preliminary data found no clear pattern of inheritance; these plants will be re-inoculated and additional seedlings tested. Marker analysis is also in progress to correlate RAPD, AFLP, and SSR markers with resistance and susceptibility and create a linkage map. These markers will be useful in resistance breeding and serve as the basis for more detailed studies of resistance mechanisms in *Vitis*.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Transformation of *Vitis vinifera* L. cvs. Thompson Seedless and Chardonnay with the Green Fluorescence Protein Marker Gene

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The green fluorescent protein (GFP) gene is a unique tool for plant transformation and breeding experiments because it permits the monitoring of gene expression in living organisms. GFP-expressing tissues can be monitored by *in vivo* fluorescence, avoiding any need for destructive testing, and the appropriate transformants can be rescued and grown to maturity. In order to study GFP expression and secretion in grapes, proembryogenic calluses originating from anthers of *Vitis vinifera* cvs. Thompson Seedless and Chardonnay were used for transformation. Calluses were co-cultivated with *Agrobacterium tumefaciens* strain EHA101 containing three gene constructs that include a gene that codes for GFP and two types of fusions of GFP with the amino and carboxy-terminal of a ribosome-inactivating protein from *Trichosanthes kirilowii*, all under the control of the CaMV 35S-promoter. At present, we have obtained 95 plants from independent transformation events but a larger number is expected, taking into account the number of embryos that are still germinating. Fluorescence has been detected in embryos and roots, leaves and stems of the regenerated plants. The transgenic plants are currently being analyzed to determine differences among the three gene constructs. This appears to be the first report of grapevine transformation with the GFP gene.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Microsatellite DNA Analysis of Argentine Torrontes

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Torrontes riojano is a white aromatic grape variety cultivated in the Northwest of Argentina that produces a dry muscat wine. Two other Torrontes, of lower enological quality, are Torrontes sanjuanino and Torrontes mendocino. The origin of the Torrontes has been the subject of speculation and efforts to relate them to European varieties have been unsuccessful. The purpose of this work was to use microsatellite DNA polymorphism analysis to identify the three cultivars and also to clarify their origin. Allelic polymorphism at 20 microsatellite loci was analyzed in several accessions each of Torrontes riojano, mendocino and sanjuanino. All three are different cultivars. We present evidence for the hypothesis that Torrontes riojano and Torrontes sanjuanino are each the progeny of Muscat of Alexandria and Criolla chica. Likelihood ratios in support of this hypothesis will be presented. A close relationship between the Torrontes and Muscat of Alexandria has been suspected but the genetic contribution of Criolla chica is a surprise.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# The Influence of Rootstock on the Performance of Chardonnay Grapevines

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Twelve rootstocks were evaluated for six years (1995-2000) in a Chardonnay vineyard in the Salinas Valley. The site was previously planted to own-rooted vines, which were infested with phylloxera, and was not fumigated prior to replanting. The soil is a Lockwood Shaly Loam with an approximate rooting depth of 1.5m. Vines were trained to bilateral cordons and spur pruned on a vertically shoot positioned trellis. Vine spacing was 2.1 x 3.4 m (vine x row). The experimental design is a randomized complete block with eight replications of the twelve rootstocks using five vine plots. The rootstocks evaluated are 1103P, 140R, 110R, 101-14M, 3309, 44-53M, SO4, 5C, 5BB, 420A, Salt Creek and Freedom. Data collected included yield, components of yield, fruit composition, growth components and mineral composition of petioles at bloom. The yield was significantly influenced by rootstock and the six-year averages ranged from 7.09 to 11.41 kg/vine. Yield separated into three groups with 1103P, 140R, 101-14M and Salt Creek being higher, and 420A and 3309 lower. Pruning weights ranged from 0.34 - 0.94 kg/vine, with 1103P and Freedom having larger weights and 420A the lowest. Fruit composition was significantly affected by rootstock due to crop load and canopy differences. Significant differences were observed in the mineral composition of bloom time petioles due to rootstock.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Effect of Pruning and Time of Application of Gibberellic Acid and Girdling on Yield, Berry Size and Fruit Composition of Melissa Table Grapevines**

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Melissa is a new, white seedless, mid-season table grape variety. It was developed and released by the USDA Horticultural Crops Research Laboratory in Fresno, CA, in 1999. This work was conducted in 1999 and 2000 to evaluate the response of Melissa grapevines to pruning and the time of application of gibberellic acid (GA) and girdling treatments. Bud fruitfulness data showed that buds at the basal portion of a cane (i.e., nodes 1 through 3) were less fruitful than those at the middle portion of the cane. Cane pruned vine produced significantly higher yield than spur-pruned vines in two consecutive seasons. GA applied at bloom at 1, 2.5, and 5 ppm reduced berry set. The data indicate that girdling and GA can significantly increase berry size and maturity. GA and /or girdling 4 weeks after bloom was not as effective as the 2-week treatments. A combination of girdling and GA treatment significantly increased berry weight, size and advanced the time of fruit maturity. GA applied at 20 ppm berry set significantly reduced bud fruitfulness (i.e., cluster per vine) in the following season.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# The Role of the Viticultural Techniques on the Aromatic Content in Sauvignon blanc

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In one vineyard planted with Sauvignon blanc located in Friuli Venezia Giulia (N-E of Italy) during the period 1995-2000, the role of the different viticultural factors involved in the "viticultural model" (genotype, growing system, bud load, fertilization, year) was investigated and their relationships with both the main plant-productive (yield, clusters per plant, cluster weight, fertility, pruning wood, plant-productive indices) and must composition parameters (bound aromatic, fraction, free aromatic fraction, cinnamyltartaric acids, phenols, flavonoids) were studied. In the "viticultural model" of Sauvignon blanc the role of the investigated factors is very diversified: in fact, some parameters as genotype, growing system, fertilization and year assume great importance, whilst the effect of bud load appears secondary. These considerations show great practical value for the wine grower who must make his own agronomical choices, modulating the viticultural parameters within the plant-productive balance of the cultivar. Besides, it was observed a significant effect of main factors on glucosides content, confirming that agronomical strategies adopted modify the flavour potential of Sauvignon blanc. The methoxyppyrazine content of the grape also assumes a primary role in defining the quality. The tests made have shown the influence of viticultural choices on methoxyppyrazine content: in particular, the effect of the growing system has been demonstrated, whilst genotype does not seem to assume particular importance. The result obtained from the evaluation of the methoxyppyrazine tends as ripening goes on has been important: as supported by international bibliography, isobutyl-methoxyppyrazine and isopropyl-methoxyppyrazine decrease during ripening, whilst ethyl-methoxyppyrazine increases considerably. The ethyl-methoxyppyrazine is the only one that has a significant correlation, both positively to the sugar content and negatively to the titratable acidity.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Evaluation of Rootstock Germplasm for Resistance to Harmony Virulent Root-Knot Nematodes

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Development of rootstocks with resistance to root-knot nematodes (*Meloidogyne* spp.) is a top priority in grape breeding. Resistant rootstocks are one approach to root-knot nematode management, but nematodes capable of reproducing on otherwise resistant rootstocks, such as Freedom and Harmony, have been identified. Resistance to these root-knot nematodes, here called Harmony virulent populations, is known from only a few sources, including the rootstock selections 10-17A, 10-23B, and 6-19B, and Ramsey x Schwarzmann selections. Additional sources of resistance to Harmony virulent root-knot nematodes are needed. Progeny testing was used to assess the breeding value of potential sources of nematode resistance. Seedling resistance was measured by counting the number of stained nematode egg masses visible per root system. Roots were stained in a solution of eosin-Y (0.25 g/L for one hour) six weeks after inoculation with approximately 1500 second stage juveniles of Harmony virulent *Meloidogyne* spp. Since several Harmony virulent nematode populations are capable of reproducing on *Vitis champinii* based rootstocks (including Dog Ridge and its descendents Harmony and Freedom), other *Vitis* species were investigated as potential sources of nematode resistance. 1613-59 is thought to derive its nematode resistance from *V. solonis*, but is closely related to both Harmony and Freedom. Seedlings of 1613-59 showed low levels of resistance to Harmony virulent nematodes. *Vitis aestivalis* has been suggested as a source of nematode resistance, although it has been more important historically in breeding fruiting varieties than rootstocks. Seedlings of two *V. aestivalis* hybrids, Lenoir and Jaeger 70, were evaluated for nematode resistance; Lenoir is not recommended for further breeding of nematode resistant rootstocks. 161-49C seedlings demonstrated only low levels of resistance. Seedlings of *Ampelopsis arborea*, a *Vitis* relative, showed high levels of resistance to a Harmony virulent root-knot nematode. The utility of this species in viticulture is currently unexplored.

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# Proton-Days™: A Metabolic Activity Indicator

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Experimental evidence suggests the diurnal cycle of electrical potential of a noble metal sensor implanted in the extracellular region of plant tissue is an indicator of the diurnal cycle in extracellular proton concentration. Proton transfers back and forth across the cell membrane are part of a wide variety of cellular metabolic processes. The magnitude of the change in proton concentration of the extracellular fluid each day can be used as an indication of the magnitude of this activity for that day. This magnitude is termed a "Proton-Day™." A typical Proton-Day™ value for vigorous vines is 180 while poorly performing vines will have a Proton-Day™ value of 65 under the same basic environmental conditions. Proton-Days™ can be added up in the same manner as "Degree-Days" to yield a seasonal numerical index for a vineyard. Furthermore, the Proton-Day™ per Degree-Day ratio forms a quantitative response/stimulus ratio for rating vineyard performance.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Rootstock and Location Effects on Chardonal Productivity, Fruit and Wine Quality

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Chardonal is an upright-growing white wine grape resulting from a cross of Seyval blanc x Chardonnay that was released in 1990. Chardonal is more disease-resistant and winter-hardy than Chardonnay and is phylloxera-tolerant. A cooperative study was initiated between the University of Arkansas and California State University, Fresno to determine the effect of rootstock on productivity, fruit composition, and wine quality. Viticultural data was collected for five years in Arkansas and three years in California and wine was made one year. Chardonal scion wood was bench grafted on Cynthiana, Freedom, Kober 5BB and Richter 110. Grafted vines and own-rooted-vines were planted in Fayetteville, AR and Fresno, CA. Vineyard establishment, trellis systems and cultural practices were standardized between locations. The two locations have different soils and climates and results differed between locations. Grapes grown in Fresno had higher Brix (23.5) and acidity (8 g/L) and lower pH (3.44) than in Fayetteville (20.5), (6.4 g/L) and (3.60). Vines on Cynthiana rootstock did not grow well at Fayetteville but performed similarly to own rooted vines at Fresno. The greatest benefits of using rootstocks were seen in Fayetteville where yield increases of 39 and 28% were obtained with 5BB and 110R respectively. Cynthiana produced the lowest yields at both locations while Freedom produced the largest vine size. Grapes from 110R had a lower pH than own-rooted vines at Fayetteville. Otherwise, there were few quality differences between own-rooted, Freedom, 5BB and 110R rootstocks. Wines from Freedom had the highest pH at both locations. There were few other differences in wine pH, acidity, or color among grapes that were not attributable to maturity. There was not a major advantage to using rootstock in Fresno. The use of 110R seems to hold an advantage in Fayetteville over own-rooted vines due to higher yield and lower pH.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Effect of Gibberellins on Induction of Parthenocarpic Berry Growth of Three Grape Cultivars and Their Endogenous Gibberellins

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Gibberellins A<sub>3</sub> (GA<sub>3</sub>), A<sub>4</sub> (GA<sub>4</sub>) and A<sub>7</sub> (GA<sub>7</sub>) were evaluated for their induction of parthenocarpy and subsequent retention of grapes ('Campbell Early', 'Delaware' and 'Koshu') berry. Gibberellins were applied to flower cluster 2 weeks before and 10 days after full bloom. These gibberellins induced parthenocarpy in three grape cultivars. However, the biological activity of the treated gibberellins was different among cultivars. In 'Campbell Early' and 'Delaware', all gibberellins were effective to induce parthenocarpic berry growth. Especially, GA<sub>7</sub> was more effective in parthenocarpic berry set and weight. In case of 'Koshu', the percentage of berry persisting was extremely lower in all applications of GA<sub>3</sub>, GA<sub>4</sub> or GA<sub>7</sub>, plus the effects of GA<sub>4</sub> or GA<sub>7</sub> were lower than that of GA<sub>3</sub>. It did not increase the percentage of parthenocarpic berry set, when high concentration of GA<sub>3</sub> was applied to 'Koshu'. When GA<sub>3</sub>, GA<sub>4</sub> or GA<sub>7</sub> were applied to flower cluster of 'Campbell Early' and 'Delaware', the pollen germination was strongly inhibited, but such a trend was not observed in 'Koshu'. Extracts from immature seeds of three grape cultivars were separated by HPLC. Using the dwarf rice seedling ('Tan-ginbozu') bioassay, GA-like activities in three cultivars were primarily confined to three fraction zones (I, II and III) on the chromatograms. Zone I was represented by fraction number 13 to 16, Zone II by 23 to 25 and Zone III by 26 to 28. The fraction zone which was detected the most biologically active gibberellin-like substances was III in 'Campbell Early', II and III in 'Delaware' and I in 'Koshu'. However, total gibberellin-like activity was higher in 'Campbell Early' followed by 'Delaware' and 'Koshu'.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Response of Thompson Seedless to Gibberellic Acid Applied at Different Phenological Stages and Concentrations

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Gibberellic acid ( $GA_3$ ) at 20 and 40 grams per hectare (8 and 16 grams per acre) was applied to Thompson Seedless for raisin production both in 1999 and 2000 at four phenological stages: bloom, seven days after full bloom, berry set, and fourteen days after berry set.  $GA_3$  applied at bloom thinned berries (berries/cm lateral) 28% and 18% in 1999 and 2000, respectively. Reduced thinning response in 2000 was attributed to cool temperatures during the bloom period.  $GA_3$  applied seven days after full bloom or later did not thin berries; clusters in these treatments were compact but rot was not increased.  $GA_3$  applied at bloom increased fruit maturity and improved raisin grade in 1999 but not 2000.  $GA_3$  applied at berry set or later delayed fruit maturity in 2000.  $GA_3$  increased fresh berry weight 18% both years; response was similar regardless of amount or time of application.  $GA_3$  lengthened berries but had no effect on diameter.  $GA_3$  applied seven days after full bloom or later generally increased fresh and raisin yield with no impact on raisin grade. When applied at bloom, fresh and raisin yields were increased in 2000 but not 1999. Vine fruitfulness was not affected by  $GA_3$  regardless of time or amount applied. Response to  $GA_3$  applied at 20 and 40 grams per hectare was similar for all parameters measured.  $GA_3$  applied between full bloom and berry set increased berry weight and length but had no effect on cluster thinning or fruit maturity.

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# Respiration Rates of 3309C Rootstock Cuttings

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Few if any measures exist of basal metabolic rates for dormant rootstock cuttings used in grapevine propagation, yet loss of carbohydrate during storage and callusing could be important. We used an ultra-sensitive carbon dioxide and oxygen analyzer to measure respiration rates for cuttings of the rootstock 3309C (*V. riparia* X *V. rupestris*). The rate of CO<sub>2</sub> respiration at 2°C averaged 16.1 ± 0.5 nmol CO<sub>2</sub> cm<sup>-3</sup> s<sup>-1</sup> (n = 21), or an average of 3.5 mg C cane<sup>-1</sup> day<sup>-1</sup>. Thus, carbon loss under a storage temperature of 2°C was negligible and would not significantly alter the carbon balance of the cane. When canes were placed in callusing media at a temperature of 32°C for one week, the respiration rate increased by nearly three orders of magnitude to 7.8 ± 0.4 μmol CO<sub>2</sub> cm<sup>-3</sup> s<sup>-1</sup>. The respiratory quotient (the ratio of CO<sub>2</sub> emitted to O<sub>2</sub> consumed by respiration, CO<sub>2</sub>/O<sub>2</sub>) was near unity (0.9 ± 0.1, mean ± SE, n = 5), and reflected that the primary carbon source consumed during the callusing period was sucrose. The total consumption of nonstructural carbohydrate was equivalent to slightly over 1% of the total cane mass. Thus, our results indicate that the callusing process is an important carbon sink, and that significant loss of reserve carbohydrate occurs.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Root Architectural Characteristics of Grapevine Rootstocks

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Root architecture describes the spatial arrangement of a root system based on geometric properties such as lateral root emergence angle, number of lateral roots per length of taproot, and lateral root length. Root architecture allows us to predict the topography of a root system in soil, and to draw inferences concerning foraging behavior. Nearly all investigations of *Vitis* rootstock topography have relied upon open pit trenches to describe root distributions. These kinds of investigations are informative, but time consuming and strongly depend on soil type and plant age. We used an image analyzing approach to characterize the principal architectural features of rootstocks important to California viticulture (St. George, 110R, 101-14 Mgt, 3309C, 5C, 420A Mgt, and Freedom). The emergence angle of laterals did not differ among these genotypes. The number of laterals per unit of taproot length and the arrangement of lateral roots along the taproot did differ, and suggested this trait helps explain the deep rooting behavior of genotypes like St. George. The diameter of emerging roots (mm) was smaller, the specific length ( $\text{mg cm}^{-1}$ ) was smaller, and the length per volume ( $\text{cm m}^{-3}$ ) was greater for genotypes like Freedom and 3309C. Thus, these genotypes may invest less carbon into root structure than other genotypes to forage from the same soil volume.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# **Use of the Natural Products, AUXIGRO® and VALERO® to Control Powdery Mildew and Other Pests In Wine Grapes**

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AuxiGro®, a plant growth enhancer, and Valero® miticide/fungicide have both been granted exemption from tolerance on agricultural commodities by the EPA and are currently registered for use in grapes and other agricultural crops. Both products have been the focus of University and grower plot evaluation for several years and have each shown utility in the management of disease and/or insect/mite pests and increased quality of yield. The use of natural products in grape production provides multiple benefits in pest management; flexibility in labor management via 4-hr REI, zero day PHI, and inherent worker safety, soft programs suitable for IPM strategies, low-impact alternatives to sulfur, and useful rotation away from synthetic products which may have resistance, residue, or impact on fermentation concerns. Data from University research and grower conducted vineyard trials will be presented demonstrating consistent control of target pests when the products are used alone or in rotation with other control options. Furthermore, AuxiGro is also registered to enhance sugar (Brix) production. Discussion will focus on methods for incorporating these low impact products into grape production systems, and usefulness in rotation with commonly used control products.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# Direct Detection of *Xylella Fastidiosa* of Pierce's Disease in Xylem Fluids of Grapevines

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For identification/detection and basic research of Pierce's disease (PD), it is important to develop a simple technique that can be used for directly detecting PD bacteria (*Xylella fastidiosa*, Xf) from the grape tissues, especially before the infected plants develop symptoms. Using a pressure chamber, xylem fluids were extracted from petioles and shoots of infected and healthy grapevines. Xfs were isolated from the xylem fluids of the shoots with PD symptoms while no bacterium was found in the xylem fluids of the healthy shoots in the same vine. PCR amplifications of specific regions among *X. fastidiosa* strains were achieved from the bacterial suspensions of xylem fluids collected by an immunological reaction or from direct centrifugation. In comparison with the traditional squeezing technique or immunological isolation of Xf from grape tissues, the new technique reported here provided a simple and fast protocol for the bacterial isolation and detection, and could be useful for the epidemiological study of Pierce's disease.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001

# The Impact of Grape Rootstock – Scion Compatibility on Propagation Success

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The development of grape rootstock varieties has a long tradition in Hungary leading to the development of the Teleki *Vitis berlandieri* X *V. riparia* hybrids, the world's most popular rootstocks. Rootstock breeding efforts continue at the Georgikon (GK) Faculty of Agriculture with emphasis on better adaptation to high lime soils. Genetic compatibility among scions and rootstocks (particularly newly developed rootstocks) is an important issue and influences propagation success. Certified scions from 11 white wine grape varieties, 5 red wine grape varieties and 6 table grape varieties were grafted on 5BB, GK59 (*V. berlandieri* X *V. riparia* hybrids), GK28 ((*V. berlandieri* X *V. riparia*) X *V. vinifera*) and GK121 ((*V. berlandieri* X *V. riparia*) X *V. rupestris*). Callus development was evaluated at the basal end of the rootstock and at the graft union for all possible combinations. No correlations were determined between basal callus development and graft success. However, callus at the graft union combined with basal callus development correlated well with graft success ( $r^2 = 0.68$ ). In general, white wine grape varieties grafted more easily than red varieties with 'Italian Riesling' the best and 'Blaufrankisch' the worst of the tested varieties. Rootstock also had a role in callus development and graft success. GK121 produced the best results, 5BB and GK28 were intermediate, and GK59 was worst and has been discarded from the breeding program. Root weights, shoot weights, overall graft success of all combinations will be discussed.

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ASEV 52nd Annual Meeting, San Diego, California, June 2001