Technical Abstracts

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Technical Abstracts

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Red Blotch Symposium

Exploring the Host-Pathogen Molecular Interactions through Multi-OMICs for GRBV Control in Grapevine

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The lack of targeted approaches to control grapevine red blotch virus (GRBV) through vector management presents a significant challenge to the wine industry. Costly vine removal represents the only effective option, underscoring the urgent need for targeted alternatives. RNA interference (RNAi) is a conserved defense mechanism in plants, triggered by the virus' presence during early infection stages. Currently, the RNAi-related molecular interactions between GRBV and grapevine are poorly documented. Establishing a solid understanding of the GRBV-host arms race is essential to develop effective control strategies that enhance the RNAi response. Our research aims to investigate these interactions, focusing on the plant's RNAi response during early GRBV infection stages. After generating a population of GRBV-infected microvine plants through *Agrobacterium*-mediated infiltration, we observed a peak of viral replicase activity one week postinfection. Subsequent small RNA sequencing revealed the presence of 21, 22, and 24-nucleotide virus-derived small-interfering RNAs (vsiRNAs), indicating RNAi gene silencing activity. Using a custom bioinformatics pipeline, we identified nine GRBV genomic regions, or hotspots, targeted by the plant's RNAi. Additionally, GRBV-targeted bisulfite sequencing revealed hypermethylation within these hotspots, peaking at 24 days postinfection (dpi), underlining a potential correlation between small RNA production and the methylation of the viral genome. Based on these findings, we evaluated hotspots-derived double-stranded RNAs (dsRNAs) to silence GRBV activity in infected tissue-cultured plants. Our preliminary data suggest a significant reduction of the viral replicase and V3 transcripts abundance at 12 and 24 dpi. Our results demonstrate the efficacy of enhancing RNAi through dsRNA application for GRBV silencing. Evaluation of other target genes is underway. To the best of our knowledge, this is the first report experimentally validating vsiRNAs produced during early GRBV-infection. Our findings underscore the potential of RNAi-based approaches, emphasizing the importance of foundational knowledge in optimizing such strategies for sustainable vineyard practices.

Funding Support: Oregon Wine Board

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Red Blotch Symposium

Scalable Vine-Level Assessment of Grapevine Red Blotch Virus Infections from Aerial Hyperspectral Images

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Grapevine red blotch virus (GRBV) poses a significant threat to viticulture, leading to substantial economic losses because infected vines must be removed to prevent further spread. In this study conducted in October 2021 within a 3-ha Cabernet franc vineyard, we employed hyperspectral remote sensing with a drone-mounted camera to enhance efficiency of red blotch virus detection. The hyperspectral camera captured images in 25 spectral bands within the visible to near-infrared (VIS-NIR) domains (520 to 820 nm).

A total of 264 vines were selected randomly and sampled for PCR analysis to confirm the red blotch virus's presence. Concurrently, images were acquired using the drone and processed through segmentation techniques to extract the vine canopy signal of the selected vines. Additionally, field experts visually inspected the vines to identify infected plants. The accuracies of both the hyperspectral images and the expert assessments were compared to the PCR results.

Six machine-learning models were trained using spectral bands as predictors. Additionally, a radiative transfer model (PROSPECT) was applied in reverse mode to predict leaf pigment concentration (anthocyanins, carotenoids, and chlorophyll) based on vine reflectance, and the output was explored as an alternative set of predictors for detecting vine infections. The overall accuracy reached 87.0% using raw spectral images and 81.4% using the PROSPECT output. The highest feature importance was attributed to the estimated anthocyanin content in leaves.

This preliminary study marks a crucial advancement toward developing an automatic system for the plant-level detection of red blotch-infected vines. Integrating hyperspectral remote sensing, PCR analysis, and machine learning techniques demonstrates promising potential for more efficient and accurate identification and management of red blotch viruses in vineyards.

Funding Support: CDFA SCBGP, CSU ARI, F3, CDFA-PDGWSS

Enology – Sensory of Grapes and Wine Session

Surveying U.S Fresh-Market Grape Consumers to Determine Key Attributes and Willingness to Pay

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Grapes (genus *Vitis*) have two subgenera, *Vitis* and *Muscadinia*. While *Vitis* (especially *Vitis vinifera*) is the backbone of the grape and wine industry, it can be challenging to grow in many U.S. states due to disease susceptibility. Current grape breeding efforts focus on introgressing disease resistance from *Muscadinia* to *Vitis* and quality traits from *Vitis* to *Muscadinia,* resulting in new wide-hybrid cultivars. In 2023, an online survey was distributed targeting U.S. consumers of fresh-market grapes to assess demographics, purchase habits/intent, and willingness-to-pay (WTP). Consumers were screened based on age (over 18-years-old), residing in the U.S., and purchase of grapes in last 12 months, with a quarter of respondents from each U.S. area (southeast, northeast, northwest, and southwest). For the discrete choice experiment, there were 24 WTP choice sets (bunch grape, muscadine grape, or neither) with five price levels per pound ($2, $3, $4, $5, and $6), flavor (strong or mild), texture (soft or firm/crisp), size (small, medium, or large), seed presence (none or present), and color (purple/black, pink/red, or light/green). Consumers (n = 950) were 51% female, averaged age 45, had income of $95,000, and were mostly white/Caucasian (86%). Consumers purchased fruit from grocery stores (89%), super discount stores (43%), direct from the farm (30%), online (20%), or at health food stores (15%). Consumers ranked fruit attributes from most to least important (freshness, flavor, price, seed presence, texture, color, size, nutritional content, production style, and shopping environment). Consumers were willing to pay more for muscadine grapes ($5.57), firm texture ($1.44), and no seeds present ($3.59), but less for pink/red color ($0.94) or purple/black color ($0.69), and consumers had no preference for fruit size. Results indicated a consumer demand for both bunch and muscadine grapes, preference for firm, green, seedless grapes, and tendency to purchase from traditional fruit purchasing channels.

Funding Support: USDA NIFA Specialty Crop Initiative (SCRI) planning grant team for "Through the Grapevines: Building Research and Extension Potential between Subgenera Vitis and Muscadinia for the U.S. Grape Industry" (Award # 2022-51181-38326)

Comparison of Pre- and Postfermentation Alcohol Manipulation of Cabernet Sauvignon Wines

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Cabernet Sauvignon grapes were harvested at three potential alcohols (11, 13.5, and 16% v/v, ABV) from a vineyard in Sunnyside, Washington in 2018 and 2019. Alcohol was controlled for pre-fermentation by either dilution or chaptalization. At each harvest, the 13.5 and 16.0% ABV wines were dealcoholized to 11 and 13.5% ABV using a pilot-scale dealcoholization (DA). The initial experimental alcohol was treated as the control, the dealcoholized wine the treatment, and the wine sharing the same alcohol target and harvest date as the dealcoholized wine was designated as the negative control. Basic wine chemistry was determined and untargeted solid-phase microextraction gas chromatography-mass spectrometry was used to identify major aroma compounds present. Targeted analysis was performed on various classes of

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Enology – Sensory of Grapes and Wine Session—CONTINUED

wine aromatics (alcohols, esters, aldehydes, terpenes, pyrazines) informed by the untargeted analysis. Descriptive analysis was performed on the 2019 wines by nine panelists (four males). Three taste, three mouthfeel, five ortho-, and seven retro-nasal aroma attributes were significant. DA had no significant affect on any basic wine chemistry other than the intended alcohol. For all aroma compound classes measured, the negative control and dealcoholized treatments were significantly lower in concentration than the higher ABV controls. Similarly, the retro- and ortho-nasal aroma attributes were rated lower intensity in the negative control and DA treatments than in the higher ABV controls. Generally, the experiment shows that dealcoholization is not absorbing or "scalping" aroma compounds. Instead, the reduction of aroma compounds is due to volatile compound partitioning effects that are directly influenced by ethanol concentration. These results show that wine aromas, and thus wine styles, may be manipulated postfermentation by dealcoholization.

Funding Support: Washington Wine Commission

Effect of Cap Management Protocols on Phenolic Composition, Redox Potential, and Sensory Properties of Pinot noir Wines

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Pinot noir wines from the Sta. Rita Hills AVA of California were produced with five contrasting cap management protocols: pump-overs, performed at two full-volumes/day (PO); punch-downs, performed twice/day for 3 min each (PD); no cap management (NoCMgmt); air mixing, at two additions/day (AM); and nitrogen mixing, at two additions/day (NM). Redox potential spikes up to 340 mV were observed during maceration in AM wines. NM wines consistently showed negative mV values with subtle -40 mV spikes during N mixing. Temperature was controlled during fermentation (24 to 28°C), trending 2°C lower in PO wines. Alcoholic fermentation was completed in all wines within 10 days, with a slower rate of total soluble solids depletion in NoCMgmt wines. PO wines had lower alcohol levels (12.76%) than PD wines (13.76%), and acetic acid doubled in AM wines. AM wines showed the lowest levels of acetaldehyde and NoCMgmt the highest. Anthocyanins and tannins were 50% lower, and total phenolics were 44% lower in AM wines. Total polymeric pigment formation was favored in PD wines at 40% higher and 42% higher relative to PO and AM wines, respectively. The tannin, anthocyanin, total phenolic, and chromatic profiles of PD and NM wines were statistically indistinguishable, but lower in NoCMgmt wines, and the lowest in AM wines. An accelerated aging experiment was established by placing the wines in air-tight glass ampoules, then incubating them for five weeks at 38°C. After accelerated aging, alcohol, pH, titratable acidity, acetic acid, tannin, and total phenolics levels were essentially unchanged and consistent with those measured at bottling. Whereas anthocyanins dropped by 70% and polymeric pigments increased by 106% across all wines, the trend described at bottling for these phenolics persisted. Sensory analysis is currently underway. Present results emphasize the well-documented subpar effects of excessive oxygen dissolution, even during alcoholic fermentation, on Pinot noir wines.

Funding Support: Star Lane and Dierberg Vineyards, LLC (Santa Ynez, CA, USA), E. & J. Gallo Winery (Healdsburg, California, USA), and Treasury Wine Estates (St. Helena, California, USA).

Enology – Sensory of Grapes and Wine Session—CONTINUED

Assessing the Effect of Grape Smoke Exposure on Different Red Wine Varietals using Instrumental and Sensory Analysis

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Fresh smoke from wildfires releases volatile phenols (VPs) due to the thermal degradation of wood lignin. VPs are absorbed into grape berries and rapidly glycosylated. Wines made from smoke-affected grapes are characterized as having smoky, medicinal aromas and a retro-nasal ashy aftertaste. Around 15 to 20% of general consumers are anosmic towards the ashy character of smoke. This study investigated the baseline levels of VPs in grapes across seven different red wine varietals spread across California and the sensory differences between the smoke-affected and non-smoke-affected wines using modified descriptive analysis (DA), gas chromatography-mass spectrometry, and liquid chromatography-triple quadrupole tandem mass spectrometry of the free and total VPs, and individual bound glycosides, respectively. The amount of smoke exposure was the largest contributor to smoke impact, but different wine matrices from different locations and varietals were also important in determining the level of perceived smoke impact. Low smoke-affected wines determined by free and total VP concentrations were not significantly different from the non-affected wines when rated by DA. The study also investigated the difference between the sensitivity of experts and non-experts towards smoke. From the same location, smoke-affected and non-smoke-affected wines made from Cabernet Sauvignon, Malbec, and Syrah grapes, respectively, were blended through serial dilutions to achieve wines with different levels of smoke impact. Wines were evaluated using a series of hedonic questions, just-about-right, and check-all-that-apply. The results indicate that experts could determine smoke impact at lower percentages of smoke-affected wine included in the wine blend than non-experts, who only distinguished smoke impact at higher percentages of smoke-affected wine in the wine blend, and thus smoke marker compounds. Research findings from both studies contribute to our understanding of grape smoke exposure and how it affects wine quality as determined by instrumental measurements, wine experts, and non-experts.

Funding Support: Jackson Family Wines, USDA-ARS

The Efficacy of Common Winemaking Practices on Reducing Wildfire-Smoke Flavor Perception in Cabernet Sauvignon Wines

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With the increasing frequency of wildfires in wine-producing regions around the world, wine producers are looking for strategies to reduce the sensory impact that these fires have on wine. Wines produced from smoke-exposed grapes have been found to have smokey, burnt, and dirty aromas and flavors, along with a distinctive lingering ashy finish. Several mitigation strategies have been studied to determine their level of efficacy in reducing the effect of wildfires on wines, looking at both chemical composition and sensory alterations. This study further evaluated the influences of common winemaking practices on the sensory properties of wildfire-affected wines. Using descriptive analysis of Cabernet Sauvignon wines, blending, carbon fining, SRX resin treatment, oak chips, and differing oak barrel usage were all evaluated. The results indicated that blending did not consistently reduce smoke

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Enology – Sensory of Grapes and Wine Session—CONTINUED

flavor perception as the percentage of smoke-affected wine in the blend was reduced. The resin treatment showed more promise for reducing smoke flavors than the carbon treatment. For oak, differences based on oak origin were observed, with greater intensities of smoke flavors with French oak than American oak. Finally, this study showed that from a sensory perspective, there was no carryover of smoke flavors from used barrels that previously held smoke-affected wine. Overall, this work showed that common winemaking practices can effectively reduce the effect of wildfire on wines; however, further trials must determine what provides the most benefit for a specific vintage. This work helps increase our knowledge of the sensory alterations that wildfires cause and how widely used winemaking practices affect wine sensory properties. With this knowledge, sensory-guided decisions can be made on what strategies to employ in future wildfire years.

Funding Support: USDA-SCRI grant no. 2021-51181-35862/project accession no. 1027470

Viticulture – Climate and Environment Session

Simplifying Environmental Sustainability Assessment for Grapegrowers: A Parsimonious Model Approach

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Environmental sustainability has become a key factor in production and consumption of many goods and services. Compulsory requirements from organizations, governments, markets, and society push industries like agriculture to assess and lower their environmental impact. The wine industry, especially the viticulture sector, faces several challenges. Vineyards are often managed intensively with herbicides, systemic fungicides, and insecticides with a high application frequency. An adequate strategy to improve sustainability of the wine industry must start with an accurate and objective quantification of its sustainability performance. Life cycle assessment (LCA) is a widely accepted tool for this. Grape production processes can vary considerably between wineries. Consequently, conducting LCA to identify potentials to improve environmental sustainability is highly context-specific, labor-intensive, and requires expertise in LCA. Therefore, it is not yet a hands-on tool for many wineries to assess sustainability of production processes. Simplifying LCA models could increase its use as a management and decision tool in the wine industry. Simplified models must consider specific regional aspects and individual management decisions, but require just a few key parameters to obtain representative results. For the remaining input data, necessary to build models with high predictive power, fixed generic data can be used. This study sought to distinguish input parameters that can be set to a fixed value from those that must be case-specific. Average input data for vineyard management and its probabilistic distribution was collected from the literature and from research and practice experts. The resulting inventory was analyzed in Brightway2 using Monte Carlo simulation and global sensitivity analysis to establish a parametrized inventory. Based on this knowledge, a simplified LCA model was developed by fixing input parameters with low relevance at their median impact values.

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Viticulture – Climate and Environment Session—CONTINUED

Mapping Global Future Potential for Pinot noir Cultivation under Climate Uncertainty using Generative AI

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This study addresses the effect of climate change on the global wine industry, specifically focusing on the suitability of regions for cultivating major international grape varieties. With the increasing challenges posed by climate change, understanding how shifts in climate may affect the quality and production of single-varietal wines is crucial. We propose using a climate-based wine variety recommendation system, using deep-coupled autoencoder networks, to predict regions that will likely undergo improvements or declines for key grape varieties. We tested this approach by predicting possible future Pinot noir regions globally. The system was fine-tuned and evaluated using vintage scorings from representative regions over the past ~30 years, using scorings from multiple respected wine critics. Future predictions are mapped using existing climate models under +2C and +4C scenarios from the TerraClimate Dataset. We use derived climate indicators to identify regions with the greatest potential for Pinot noir. Our findings indicate significant shifts in the suitability of regions, particularly in areas previously considered too cold. This study demonstrates the practical application of wine-recommendation systems in adapting to changing climate conditions and provides valuable insights for the wine industry. By fine-tuning these systems for specific tasks, such as predicting suitable regions for a specific varietal, the wine industry can proactively address the challenges posed by climate change and make informed decisions for sustainable viticulture. This research highlights the wide-ranging possibilities of wine-recommendation systems, showcasing their potential to enhance decision-making processes within the wine industry amid evolving climate conditions.

Funding Support: This research was supported by funds from the Canada Graduate Scholarships-Master's (CGS M) Program by the Natural Sciences and Engineering Research Council of Canada held by JH.

Role of Grapegrowers in Pollinator Conservation: Protecting and Promoting Bees within Vineyards

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Bees are essential pollinators of many crops and wild plants, thus holding great economic and environmental importance. However, research has shown declines in various bee taxa due to several threats including habitat loss, climate change, pesticide exposure, and interactions with non-native species. Successful pollinator conservation actions must be determined in lands where threats are likely to occur. This research should be done through collaboration with the land managers responsible for their implementation. This is especially needed in crops that are understudied and underused for their potential pollinator conservation capacity, such as those that are pollinator-independent like the winegrape, *Vitis vinifera*. This research project aims to determine how wild bees can be best supported in Canadian vineyards. Twenty-four commercial vineyards across the Niagara Region, ON, were surveyed to determine the effects of various vineyard management practices and surrounding landscape factors on wild bee abundance and diversity.

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Viticulture – Climate and Environment Session—CONTINUED

Bee communities were sampled monthly throughout the spring and summer using pan traps and netting for two years. The vineyard management practices under investigation included cover cropping, mowing frequencies, and organic versus conventional versus certified sustainable management. Surrounding landscape factors included proximity to and percentage of natural lands, impervious surfaces, and different crop types. Bee responses to these variables will be discussed, as well as future directions including engaging grapegrowers in pollinator conservation and important policy implications.

Funding Support: York University, Entomological Society of Canada

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Trans-Resveratrol Derived from Cabernet Sauvignon Pruning Waste: An Additional Revenue Source for Grapegrowers

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This study explores the use of trans-resveratrol from Cabernet Sauvignon grape cane waste in California as an innovative and sustainable income stream for vineyard growers. Employing high-pressure liquid chromatography, we quantified trans-resveratrol concentrations to assess the efficacy of different ethanol extraction concentrations, vineyard location, and soil composition to optimize yield. This process has a twofold advantage: enhancing profitability by converting pruning waste into valuable antioxidants and promoting environmental sustainability by decreasing agricultural waste. The investigation determined that using 50% ABV ethanol significantly surpasses alternative methods for extracting maximum trans-resveratrol yields, demonstrating the economic and environmental benefits of this sustainable practice.

Comparative analysis of five distinct vineyards in California reinforced the necessity of careful site selection and the adjustment of extraction parameters to boost trans-resveratrol extraction. This approach provides not only a method to increase vineyard revenue, but also to advance sustainable agricultural practices by transforming waste into a resource.

Converting agricultural waste into antioxidants like trans-resveratrol provides a viable pathway for vineyards to enhance their economic model while contributing to ecological conservation. Ethanol extraction of 50% ABV was the superior technique, providing a practical framework to reduce environmental footprint and advance waste to wealth strategies in agriculture, promising notable gains for the economy and the environment.

Funding Support: Dr. Qun Sun

Viticulture – Irrigation Management Session

Response of Riesling Grapes to Temporally and Spatially Heterogeneous Soil Water Availability

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Regulated deficit irrigation (RDI) and partial rootzone drying (PRD) have produced diverse viticultural and enological outcomes when implemented in vineyard settings around the world. Most research on deficit irrigation strategies has focused on red wine grape cultivars, optimizing attributes important for red wine production, which differ from white winemaking. A three-year field trial was conducted in southeastern Washington with Riesling winegrapes to compare the effect of RDI and PRD with a no-stress control and test their suitability for premium white wine grape production in arid climates. Irrigation scheduling was based on soil moisture thresholds according to vine phenological stage, rather than adhering to specific time intervals. Irrigation water supply, soil and plant water status, canopy size, yield components, and fruit and wine composition data were collected. Midday leaf water potential (Ψ_{leaf}) remained stable near -0.7 MPa until the extractable soil water content declined to ~35% (field capacity is at 100%). Beyond this point, Ψ_{leaf} declined as soil moisture decreased further, dropping to values as low as -1.5 MPa. Both RDI and PRD conserved irrigation water compared to the control, but both deficit practices also reduced yield. Seasonal variation had a more pronounced effect on basic fruit composition than irrigation practices. Preveraison water deficit, even if slight, reduced canopy size and yield while significantly affecting the volatile composition of the resulting wines. The wine phenolic composition was affected to a lesser extent by irrigation. This research confirms the power of small differences in preveraison water status to manipulate wine style in the vineyard and explores the relationship between the available soil water content and vine water status, using Riesling as a model.

Funding Support: USDA-NIFA Cyber-Physical Systems Program, Washington State Grape and, Wine Research Program, Chateau Ste. Michelle Distinguished Professorship.

Metabolomic Response of *Vitis vinifera* and Interspecific Hybrids to Water Deficit and Heat Stress

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Recent projections of increased temperatures linked to greenhouse gas emissions suggest an increased incidence of heat waves in Canada by 2100. In this context, vineyards will likely face more heat stress along with drier conditions, causing water deficit at critical periods of their development, including when new plots are established.

Interspecific hybrids varieties are issued from complex breeding programs involving *Vitis vinifera* and other *Vitis* species such as *Vitis riparia* and *Vitis labrusca*. In the context of global changes, interspecific hybrids bred for challenging environments such as cold climates are often expected to show greater resilience to stress than *V. vinifera* varieties. However, this assumption has been little studied. To fill this gap, we compared the metabolic response of young *V. vinifera* cv. Cabernet franc and Riesling and interspecific *Vitis* spp. Marquette and Vidal to heat stress, mild water stress, their combination, and a control under unstressed conditions over 21 days.

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Viticulture – Irrigation Management Session—CONTINUED

Leaves were sampled, extracted, and analyzed by liquid chromatography-mass spectrometry-Orbitrap.

Over 200 metabolites were detected, including amino acids, phenolic compounds, and sugars. Depending on the variety, between 50 and 70 metabolites increased significantly in plants subjected to heat, water deficit, or combined stress treatments. Heat stress generated the strongest response, but combined stress provided mixed results among varieties. For instance, most metabolites upregulated by heat stress in Cabernet franc increased further when plants were exposed to combined stress. In Marquette and Vidal, most upregulated metabolites showed a lower response during combined stress. These results suggest that the stress mitigation mobilized fewer metabolic resources in Marquette and Vidal than in Cabernet franc, which could relate to a higher resilience in these varieties.

Funding Support: NSERC; AAFC; CGCN

Effect of Irrigation Timing and Intensity for Heatwave Mitigation in Cabernet Sauvignon Grapes and Wines

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Extreme heat and drought events are becoming increasingly frequent and constitute a threat to grape production. Heatwaves are defined as two or more days with maximum temperature >38°C, and are known to negatively affect vine performance, yield, and wine quality. We conducted a meta-analysis to link historic climate, yield, and grape composition data across five ranches in the Napa region to approximate heatwave effects at the commercial scale. Cultural practices are needed to effectively mitigate heatwaves in the vineyard; in this case, increased irrigation during heatwaves was studied in Cabernet Sauvignon. Irrigation intensity (50% or 100% more than the control) and timing (zero, one, or two days prior to the heatwave) were evaluated. Experimental treatments were compared to the control: deficit-irrigated at 80% crop evapotranspiration through the heatwave. The trial was conducted in California at two sites, Lodi and Sonoma. The 2022 season was characterized by two postveraison heatwaves, one in mid August (four to five days, T_{max} = 40.2°C) and one in early September (six to nine days, T_{max} = 46.5°C). Across the two sites, additional irrigation effectively improved plant water status and physiological activity. Berry dehydration rates were significantly decreased by the most irrigation (9%) compared to the control (up to 22%), resulting in larger yields at harvest. Additional irrigation mitigated rapid total soluble solids increases observed in the control (up to 6 Brix/week). Incremental increases in irrigation levels during heatwaves resulted in wines with reduced levels of heat damage. The concentration of negative aroma compounds was greater in heat-affected wines, and that of positive fruity esters was less. Phenolic compounds were greatly affected by heat, which translated directly into lower color intensity, higher hue, and lower concentration of mouthfeel-related phenolics.

Funding Support: E. & J. Gallo Winery

Viticulture – Irrigation Management Session—CONTINUED

Precisely-Timed Irrigation Pulses can Reduce Berry Cell Death and Late-Season Dehydration

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Hot, dry conditions can exacerbate late-season berry dehydration, reducing yield and altering berry sensory properties. Late-season dehydration occurs when the berries undergo cell death in the mesocarp and the water released from the ruptured cells evaporates through the skin or is drawn from the fruit to the canopy by a water potential gradient (backflow). Here, we tested whether short pulses of increased irrigation would be a water-use efficient strategy to reduce late-season dehydration by interrupting stress-induced signals for berry cell death. We compared three irrigation treatments: conventional (following standard commercial practices) and an early- and a late-pulse treatment, where irrigation was increased by 40% in the two weeks immediately before or after the expected onset of cell death. We measured mature Cabernet Sauvignon vines grafted to 101-14 in an experimental vineyard at UC Davis in summer 2022 and 2023. We monitored vine water stress, berry cell death and shrivel, reactive oxygen species (H_2O_2) concentrations, and markers for cell oxidative damage (malonyldialdehyde, MDA). The late-pulse treatment significantly reduced the rate of cell death and the magnitude of berry shrivel at harvest over the conventional treatment. However, the early-pulse treatment did not significantly affect the rate or date of onset for cell death or shrivel. Concentrations of the reactive oxygen species H_2O_2 and markers for oxidative damage to cell membranes (MDA) increased at the same time as cell death, consistent with a role in programmed cell death, but were not significantly different among treatments, indicating that other mechanisms regulate irrigation effects on berry cell death. Overall, these findings show that the onset of cell death is not affected by water status, but a short pulse of irrigation soon after onset can slow the rate of cell death and reduce berry shrivel at harvest.

Funding Support: American Vineyard Foundation

Extreme Drought Depresses Vine Growth and Yield Regardless of Fruit Removal

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Frequent drought episodes during fruit development threaten sustainable production of premium winegrapes in the western United States. During an extreme drought event, the irrigation water supply to vineyards may be shut off and growers often remove fruit to ensure vine survival. This study investigated the idea that fruit removal during drought enhances vine survival in Cabernet Sauvignon and Riesling. In 2022 and 2023, irrigation water was shut off either at fruit set or at veraison, and 0, 50, or 100% fruit was removed at the start of each drought period. The outcomes were compared with standard regulated deficit irrigation (RDI), with and without fruit removal. Drought from fruit set or veraison lowered the vines' water status alike, and the effect was greater in vines with no fruit removed in both cultivars. Canopies of vines exposed to drought stress from fruit set were up to 5.6°C warmer than the RDI vines. Drought starting at fruit set depressed yield more than drought starting at veraison in both cultivars. Vines exposed to drought from fruit set had more than

**indicates corresponding author*

Viticulture – Irrigation Management Session—CONTINUED

45% (Riesling) or 60% (Cabernet Sauvignon) less yield than RDI vines. Drought and fruit removal had no clear influence on fruit composition. Compared to the RDI vines, there was more than a one-third reduction in early-season shoot growth and a 40 to 50% drop in the number of flowers in vines exposed to drought stress the previous year, even if fruit had been removed from those vines. Our findings demonstrate that extreme drought will have a long-lasting effect on vine growth and productivity, regardless of fruit removal.

Funding Support: USDA Northwest Center for Small Fruits Research

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Enology – Microbiology Session

Digging deeper into Microbial Terroir: Biogeography of *Hanseniaspora* in Oregon's Willamette Valley Wine Region

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The apiculate yeast genus *Hanseniaspora* has appeared frequently in enological research for over a century, mostly focused upon the species *Hanseniaspora uvarum* because of its notable capacity to cause spoilage. Recently, there has been more research into the potential benefits of other *Hanseniaspora* species, such as *Hanseniaspora vineae*, in producing more complex wines. Furthermore, large-scale, DNA sequencing-based (metabarcoding) vineyard ecology studies have suggested that *Hanseniaspora* species may not be evenly distributed. Does this mean that fruit from different vineyards could yield spoiled or complex wines depending on which *Hanseniaspora* species are present? To address this, we sampled extensively from 12 vineyards within the Willamette Valley AVA, across two sub-AVAs (Eola and Yamhill). We then used metabarcoding to assess the contribution of *Hanseniaspora* to the grape berry fungal community and their relative abundance in cold-soak and early stages of fermentation.

Our results show that while six of the 23 recognized *Hanseniaspora* species were present on Pinot noir grapes in the Willamette Valley AVA, differences between vineyards were driven by the abundance of *H. uvarum*. On the other hand, fungal community differences between sub-AVAs were not related to *Hanseniaspora* species. We observed significant positive correlation between the amount of *H. uvarum* present in must and at cold-soak, and then cold-soak to early fermentation. While intuitive, it is worth noting that no prior studies have observed this across so many grape samples from different vineyards. Our results provide clear evidence that the amount of *H. uvarum* on grapes may be an important predictor of potential effects on wine quality, particularly if performing cold-soak, which acts as an enrichment step. *H. uvarum* isolates have been recovered from almost every must sample for whole genome sequencing and are being evaluated for genetic and phenotypic differences to determine whether vineyard and/or sub-AVA populations vary.

Funding Support: Oregon Wine Research Institute, Agricultural Research Foundation and Oregon State University

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Enology – Microbiology Session—CONTINUED

Saccharomycodes ludwigii Yeast Derivatives: Novel Potential Bio-Adjuvants for the Winemaking Process

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To promote more sustainable production methods and greater respect for the natural characteristics of wines, this study evaluated the impact of non-conventional yeast derivatives on the chemical and physical properties of wines. In particular, various types of yeast derivatives obtained from *Saccharomycodes ludwigii* were used. This is an osmophilic yeast whose affect on wine has so far been evaluated only as a function of its metabolic activity. However, recent studies have indicated that yeasts belonging to the *Saccharomycodes* genus can release significant amounts of polysaccharides, particularly mannoproteins, into the medium both during growth and after inactivation. These macromolecules are known to interact with various compounds in wine, contributing to its colloidal stability. Thus, after optimizing the growth conditions favoring production of *S. ludwigii* biomass, innovative techniques for yeast inactivation were employed and the effect of different derivatives on the final chemical and physical characteristics of white wine was assessed.// All fermentation tests conducted with *S. ludwigii* derivatives exhibited better fermentation kinetics than the control. Basic parameters were consistent across all wines. However, there were significant differences in the aromatic profiles. Wines treated with *S. ludwigii* derivatives showed higher polysaccharide content compared to the control. Further analysis is warranted to evaluate the influence of these macromolecules on wine colloidal stability and sensory characteristics over time.

Funding Support: Enartis

Effect of Malolactic Fermentation Timing and Cold Soaking with *Torulaspora delbrueckii* on Pinot noir Sensory Properties

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This study investigated the influence of malolactic fermentation (MLF) timing on Pinot noir color and sensory properties. The addition of a non-*Saccharomyces* yeast, *Torulaspora delbrueckii*, during cold soak was also investigated due to potential interactions with the malolactic bacteria and production of acetaldehyde. Pinot noir wines were produced with and without cold soaking, with and without *T. delbrueckii*, and with a concurrent or sequential MLF. Cold soaking with *T. delbrueckii* yielded higher acetaldehyde concentrations at the end of cold soak and during alcoholic fermentation, but did not result in significant color differences at the end of MLF. Concurrent MLFs decreased color density and polymeric pigment content, but only if a cold soak had also been undertaken. After nine months of aging, differences in wine color and polymeric pigment content noted at the end of MLF were no longer present. Wines were assessed by a sensory panel to determine liking and sensory characteristics. Panelists were asked to rate their liking of each wine in clear and opaque glasses and to complete rate-all-that-apply descriptive analyses for aroma, taste, and mouthfeel. Wines produced without cold soaking had a significantly different aroma than those produced with a cold soak, while non-cold soak wines were significantly different from each other due to MLF timing. When not paired with cold soak, the concurrent MLF wine was described as having woody and spiced aromas, while the sequential MLF wine was described as having cedar, fruity, and herbal aromas. Use of *T. delbrueckii* during cold soaking resulted in wines described

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Enology – Microbiology Session—CONTINUED

as having a more balanced and softer mouthfeel than their counterparts. Overall, MLF timing did not affect color once wines had been aged, but did result in wines with different aroma and mouthfeel properties. Cold soaking and use of *T. delbrueckii* also resulted in wine sensory changes.

Funding Support: Oregon Wine Board, American Vineyard Foundation

Revealing Aromatic Thiols in Red Wines

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Volatile thiols are a well-known family of aroma compounds that, even at low levels, can have a significant affect on the fruity quality of wines. 3MH, 3MH-A, and 4MMP are some of the more well-known aromatic thiols. These compounds confer grapefruit, passion fruit, and boxwood notes in thiolic white and rosé wines when revealed from their odorless form. However, thiol precursors are now known to be present in important red grape cultivars (e.g., Syrah, Cabernet Sauvignon, Merlot, Pinot noir). When revealed, the descriptors used to describe the same thiolic compounds in red wines are black fruits, red fruits, and spice. The aromatic profile of thiols in red wines is complex and is thought to be due to interactions between thiols and other molecules, such as terpenes and esters.

The enzymatic action of yeast is essential to cleave the non-volatile, odorless precursors and release the volatile, aromatically active thiols. Using a cell division approach, we selected a new *Saccharomyces* yeast with the full potential for thiol revelation due to its highly efficient β-lyase enzyme activity. This enzyme activity is due to the yeast possessing the correct form of the IRC7 gene. The sequence of the IRC7 gene is not identical among all yeasts. Some yeasts have a truncated version of this gene, missing 38 base pairs. This version encodes a non-functional enzyme. Other strains can have a point mutation, close to the enzyme's active site, which has a strong negative effect on its activity.

For maximum β-lyase activity, the yeast must possess the right combination of the gene, which is the long, unmutated form (IRC7L/L Ref/Ref). This new yeast strain shows very significant thiol revelation, well above average concentration, with a very significant increase in aromatic freshness and black and red fruit notes.

Funding Support: Lallemand Oenology

Is Complex Nutrition More Advantageous than Mineral Nitrogen for the Fermentative Capacity of *Saccharomyces cerevisiae*?

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During alcoholic fermentation, nitrogen is an essential nutrient for yeast as it plays a key role in sugar transport and biosynthesis of wine aromatic compounds (thiols, esters, and higher alcohols). The main issue of a lack in yeast assimilable nitrogen (YAN) in winemaking is sluggish or stuck fermentations that promote growth of alterative species and lead to economic losses. However, correcting the N deficiency is sometimes not enough to restore proper fermentation performance. This suggests the existence of other nutritional shortages.

Enology – Microbiology Session—CONTINUED

The aim of this work was to study the effect of the timing and the nature of nutrient addition (mineral N [100% yeast derivative] or mixed complete [mineral N and yeast derivative]) on alcoholic fermentation. First, 16 commercial strains were inoculated into Sauvignon blanc grape must deficient in YAN (110 mgN/L) and with reducing sugars concentration adjusted to 240 g/L (potential alcohol content of 14.3% vol.). Fermentation kinetics of strains were then classified into three groups: stuck, sluggish, or complete alcoholic fermentations. New experiments were carried out in the same grape must supplemented in YAN with ammonium (mineral) or yeast derivative products (100% organic or mixed) to get 200 mgN/L. Nutrient additions were made at the beginning of alcoholic fermentation (single addition) or in two additions (50% at the beginning + 50% at the middle of alcoholic fermentation).

Our results show that supplementation with mixed nutrients was more beneficial for fermentation performance than mineral N alone. Fractionated addition was also more effective than single addition. This study highlights the need to think about yeast nutrition from a holistic perspective (N and lipid addition, timing of addition).

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Effect of *Pediococcus* on Wine Chemical and Sensory Properties

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Pediococci have been isolated from wines worldwide, but their specific effect on wine quality is not well described. Due to warmer growing seasons leading to higher-pH grapes and winemakers looking to reduce sulfur dioxide (SO_2) use, the risk of *Pediococci* growth in wines is increasing. Therefore, this project aimed to characterize the sensory impact of *Pediococcus* on wine and investigated the effect of wine lees and nutrient additions on biogenic amine (BA) production. Nineteen *Pediococcus* isolates from Oregon, Washington, and California wines were inoculated into sterile filtered Pinot noir and growth was observed for 56 days, followed by the addition of SO_2 and bottling. Differences in growth were observed, with many isolates maintaining a population similar to the initial inoculation rate (105 CFU/mL), while others declined in viability before recovering to higher populations. Experienced wine tasters using a modified napping method and ultra-flash profiling assessed the wines. No strong groupings of treatments based on aroma were observed, but there were two distinct groupings based on mouthfeel. One grouping was described as "Light," "Thin," and "Sharp," while the other group was described as "Sticky," "Medium," and "Sharp." These descriptors will be used to define additional chemical analysis. To explore factors affecting BA production by *Pediococcus*, different nutrient additions were made to Chardonnay juice prior to fermentation. The three nutrient addition treatments were control, high DAP, and high organic N. After completion of alcoholic fermentation, wines were cold-settled prior to racking (no-lees) or no racking (lees) treatments. Dimethyl-dicarbonate was added to kill background microorganisms and after 48 hrs, two BA-producing *Pediococcus* strains were inoculated into the wines. Growth was monitored for 60 days, after which samples were collected for analysis. BA will be assessed by liquid chromatography-mass spectrometry to determine the effect of nutrients and lees aging on BA production by *Pediococcus*.

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Enology – General Enology Session

Anthocyanin Composition: A Closer Look at Mono- and Di-glucosides

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Anthocyanins are water-soluble compounds belonging to the flavonoid family, synthesized via secondary metabolic pathways in plants. In addition to their role in red wine color and color density, there is strong evidence that anthocyanins have important antimicrobial, anti-inflammatory, and antioxidant properties. Anthocyanin stability is influenced by many factors, including pH, temperature, ultraviolet light, oxygen, enzymes, and co-pigmentation. Sugar molecules can reduce degradation of unstable anthocyanin intermediates into phenol and aldehyde acid molecules. The presence of two sugar molecules in anthocyanin structures (di-glucosides) increases their stability more than having only one sugar molecule (mono-glucosides). Anthocyanin stability is further enhanced by increased methylation and decreases as more hydroxyl groups are added to the B-ring of anthocyanidins. Therefore, it is imperative to monitor the composition of anthocyanins to better understand color stability and its contribution to wine quality. The present analytical method, implemented on an Agilent high-performance liquid chromatography instrument with diode-array detection, provides an opportunity to quantitatively measure five mono-glucosides (delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, and malvidin 3-*O*-glucoside) and five di-glucosides (delphinidin 3,5-di-*O*-glucoside; pelargonidin 3,5-di-*O*-glucoside; peonidin 3,5-di-*O*-glucoside; malvidin 3,5-di-*O*-glucoside; and cyanidin 3,5-di-*O*-glucoside) of anthocyanins in grape and wine. Analysis of seven *Vitis vinifera* varieties (Cabernet Sauvignon, Merlot, Petit Verdot, Petite Sirah, Teroldego, Pinot noir, and Zinfandel) established the dominance of mono-glucoside anthocyanins in all varieties. However, Napa Valley Cabernet Sauvignon contained elevated amounts of cyanidin 3,5-di-*O*-glucoside (up to 94.5 mg/L) and peonidin 3,5-di-*O*-glucoside (up to 78.9 mg/L). These results provide the potential to produce wine with improved color stability.

Funding Support: E & J Gallo Winery

A Second Look at What We "Know" about Tannins in Wine and How They Are Perceived

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Despite significant advances in tannin research, we cannot consistently predict tannin sensory characteristics (e.g., roughness, drying, silkiness) in all wines or predict their extraction, retention, and elongation during vinification with any certainty or reproducibility. Some of these issues result from oversimplification of a complex system. From both a perceptual and a winemaking perspective, tannin interactions are often explained using the protein precipitation model, where all proteins (and tannins) are assumed to behave similarly, with any protein equally precipitating any tannin. However, protein and tannin structures do greatly matter. While specific proteins like F6HAU0 (a vascular invertase protein) and A0A438IRV9 (ß-fructofuranosidase) are reduced in wine by tannin additions, other prevalent proteins, such as those involved in *Vitis* lipid transfer, correlate poorly with tannin retention. Similarly, human saliva interacts with a different profile of tannins than

Enology – General Enology Session—CONTINUED

common reagents used in precipitation assays (e.g., bovine serum albumin). This explains why, while these assays correlate with astringency, they often cannot explain sensory differences. Studying astringency in another high-phenolic product, cocoa, we found that test subjects perceived astringency differently and the interaction of specific salivary proteins with phenolics varied greatly by individual. These findings reinforce the idea that the complex structural differences within the tannin and protein categories contribute to their functionality. To better characterize tannin structure diversity, we have developed liquid chromatography with tandem mass spectrometry methods that can measure intact fragments of complex procyanidins post-chromatographic separation as well as elongation products caused by ethylene bridging. This comprehensive phenolic fingerprint allows us to differentiate wines and other high-phenolic foods with similar total phenolic concentrations. In the future, we expect to resolve the uncertainties in tannin evolution during winemaking and tannin perception by characterizing the diversity within tannins, proteins, and polysaccharides.

Funding Support: PA Wine Marketing and Research Program- Pennsylvania Liquor Control Board

Effect of Alternative Packaging on Vignoles (*Vitis* Hybrid) White Wine Quality during Storage

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The sustainability of grape (*Vitis* species) wine production is affected by cost and supply-chain issues with glass packaging. Although ideal as a moisture and oxygen barrier, glass wine packaging contributes one-third of the total carbon footprint for wine production. Other packaging materials, like aluminum and plastics, are used as alternatives to glass, but consumers may view them as lower quality. The effect of wine packaging on color and total phenolics of Vignoles (*Vitis* hybrid) white wine was evaluated at 0-, 6-, and 12-months storage at 15°C. Grapes were harvested in 2022, produced into wine, and bottled in January 2023. Eight wine packaging treatments were evaluated: three of glass (250, 375, and 750 mL) and five 250-mL alternatives made of aluminum, polyethylene terephthalate (PET), high-density polyethylene (HDPE), low-density polyethylene (LDPE), and polypropylene (PP). The packaging × storage interaction was significant for L*, hue, chroma, brown color, and total phenolics. For wine at 0-month compared to 12-months storage, all packaging treatments except glass (375 and 750 mL) had decreased L* and hue and increased chroma and brown color. All packaging treatments had decreased total phenolics. From 0- to 6-months storage, wine in LDPE, HDPE, and PP had >100% more brown color, while wine in aluminum and PET had 28 and 45%, respectively. From 0 to 12-months storage, wine in LDPE, HDPE, and PP had >200% more brown color; wine in aluminum and PET had 176 and 149% more brown color, respectively; and wine in glass 250, glass 375, and glass 750 mL had 68, 0, and 1% more brown color, respectively. Wine in traditional glass bottles (375 mL and 750 mL), 250 mL glass, aluminum, and PET had the most potential for further investigation of the effect of packaging on wine quality.

Funding Support: Southern Region Small Fruit Consortium

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Viticulture – General Viticulture Session

Optimizing Nitrogen Supply for Winegrape Quality in Dry Regions

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Grapegrowers in dry regions face challenges with low nitrogen (N) in harvested grapes, affecting winemaking due to insufficient yeast assimilable nitrogen (YAN). An imbalance in N supply can decrease yield or increase vine vigor, impacting grower profits. Foliar N application at veraison may enhance grape and wine composition without inducing excessive vigor, yet its effect on phenolic compounds remains unclear. We conducted a three-year field trial and two separate pot experiments in arid eastern Washington to test whether foliar-applied N moves to the fruit and other plant organs, enhancing berry ripening and quality, and potentially replenishing the available N pool to support next year's crop. In a field trial with own-rooted Syrah, we applied liquid urea ammonium nitrate (0, 22.5, 45, or 90 kg N/ha) to the soil at bloom and foliar urea at veraison. We also tested the effect of an extra foliar N spray at veraison (40 g urea/L) on potted Cabernet Sauvignon vines that had received four different rates of soil N at bloom (0 to 3.75g N per pot). Finally, using potted Riesling vines, we applied ^{15}N-labeled urea at veraison on whole canopies, leaves only, or clusters only, to trace N partitioning at harvest.

Foliar N application at veraison significantly increased YAN in field-grown Syrah and potted Cabernet Sauvignon. Foliar N supply was particularly beneficial for vines with low N status. Higher YAN in harvested berries correlated negatively with skin tannins, but not seed tannins. N applied on Riesling fruit remained in clusters, while N applied on leaves was translocated to the perennial plant organs for storage.

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Changing Perceptions of Cluster Thinning in Willamette Valley Pinot noir Production

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Cluster thinning is common in winegrape production and was conducted historically in Oregon Pinot noir to hasten ripening and ensure quality. Industry standard yield targets in the early 2010's were 4.5 to 6.2 t/ha, and growers questioned those thresholds. To systematically evaluate the effect of cluster thinning on fruit composition and wine sensory perception, a research project was conducted by Oregon State University and >20 vineyard and winery companies from 2012 to 2021. The impact of this research was quantified using a series of surveys, interviews, and focus group meetings to gather information about individual, company, and industry-wide changes to yield management practices. Project collaborators increased yield targets, as there were no consistent differences in berry ripeness at harvest and a lack of differences in wine quality between crop thinning treatments. There were no vine health consequences of maintaining higher yields (dormant pruning weight or vine nutrient status). By the mid-point of the study, the industry began adjusting yield targets based on season variability, rather than a constant tonnage/ha. This was due to the observation that wine quality was more influenced by vintage variation and winemaker than by vineyard crop thinning practices. Most collaborators reported

Viticulture – General Viticulture Session—CONTINUED

little-to-no difference in wine sensory perception between crop thinning levels. Although most project collaborators felt comfortable increasing yields by 1.24 to 7.71 t/ha without compromising quality, obstacles such as winery capacity and sales limited further adoption for some. Often, target yields were set to fit winery and sales capacity, leading to target yields that did not reflect vineyard yield-quality potential. Project collaborators improved management and vineyard knowledge and expressed a desire to share findings. The project had industry-wide impact with greater yields reported at the state level and greater tolerance of increasing yield targets by growers and winemakers alike.

Funding Support: Northwest Center for Small Fruits Research

Diabetic Grapes: Motherlode of Sugary Metabolites, Yet Unsuitable for Winemaking

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Sugars are essential to grapevine growth and development and are therefore monitored under commercial growing conditions to ensure grapes accumulate optimum sugar levels for winemaking. Leaves produce sugars, then they are translocated as sucrose via the phloem, and unloaded in berries to a maximum accumulation of 25 Brix as hexoses at veraison. Any increase or decrease in this level reflects perturbations in the ripening process. In this study, the sugar levels in four cultivars trained to vertical shoot positioning (Syrah, Cabernet franc, Sangiovese, and Petit Verdot) were much greater than 25 Brix. The sugar level in Syrah was >45 Brix, whereas in the other cultivars, it ranged from 27 to 34 Brix. This study's objective was to determine what caused such high sugar concentrations in these varieties and to provide plausible explanations. Afflicted and healthy clusters were sampled for chemical analysis of primary and secondary metabolites. The high concentration of sugars; other primary metabolites such as acids; and secondary metabolites such as anthocyanins, flavanols, and flavonols; resulted from a physiological disorder known as bunch stem necrosis (BSN). The nutritional status of the berries and the bunch stem was altered, reflected by the levels of macro- (N, P, K, Ca, Mg, S) and micro- (Fe, Mn, Zn, Cu, Mo, B) nutrients. The necrosis typically occurs on the rachis during ripening, causing dehydrated, raisin-type berries. Although BSN caused the raisining of the berries in the 2023 season, the necrosis of pedicels dominated the rachis necrosis. Cell viability tests revealed that most cells were dead. Tylose occluded most of the xylem vessels in the necrotic rachis. Although the occurrence of BSN has been known for several years, its causal factors remain obscure. This study proposes potential causal factors gleaned from the nutritional and compositional analyses.

Funding Support: N/A

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Enology – Aroma Compounds Session

Influence of Grain Type and Surface Area on Extraction of Alternative Oak in a Model Wine Solution

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One advantage of using alternative oak products is more rapid extraction than from traditional oak barrels, which is typically attributed to increased surface/volume ratio, without regard to grain type (cross- versus end-grain). This work evaluated how surface area (SA) and grain type influence extraction of oak aroma compounds. Medium plus convection-toasted staves were cut sequentially in half by length (25.4, 12.7, 6.35, 3.18 and 1.59 cm), resulting in treatments with the same total weight and volume of oak, but increased SA and end-grain to cross-grain ratio with shorter segments. All treatments used a dosage rate of 20 g/L and were extracted in model wine (15% v/v ethanol) for 240 days. Samples were analyzed using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS SPME GC-MS) to determine concentrations of furfurals, guaiacols, and lactones.

If the increased extraction rate in oak alternatives is due only to increased SA, then the extract concentration should increase proportionally between treatments with the SA increase. Instead, the extraction rate and the concentration increased as both the SA and the end-grain to cross-grain ratio increased. When the shortest segments are compared to the longest segments (45% more SA) at day 10 of the extraction, concentration increases of 130% to 15,000% (varying by compound) were observed. This suggests that increased exposure to end-grain is more important to rapid extraction than SA, as the additional end-grain accounts for at least two-thirds of the additional extraction from shorter segments. A first-order kinetic model for each compound at each SA and grain ratio was fitted to the raw data using the differential evolution parameter estimation routine (Peterson and Ulrich 2011). The outcome of this model supports the importance of grain ratio on both extraction rate and concentration.

Funding Support: Scott Labs

Investigation of Varietal Effects on the Perception of Frost Taint in Cabernet Sauvignon Wines

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Cabernet Sauvignon wines that had frozen dead Cabernet Sauvignon (CS) or Riesling (WR) leaves added at two dosages (2 or 4 g/kg must; low and high) prior to fermentation in 2022 were analyzed chemically and subjected to descriptive analysis. Basic wine analysis showed no significant changes to wine chemistry attributable to the treatment. Targeted solid-phase microextraction gas chromatography-mass spectrometry (SPME GC-MS) of volatile compounds revealed significant changes in compounds related to grassy, tropical, and citrus aromas in the treated wines. Treatments with high dosages of frozen CS leaves had significantly more 3-hexen-ol (grassy aroma) in a dose-dependent fashion and several fruity esters, phenylacetaldehyde and citronellol. Wines made with frozen leaves from WR also differed from the control in most volatile aroma compounds, but typically at significantly lower concentrations than in the larger CS frozen leaf additions. Descriptive analysis using nine panelists (five males) rated 13 ortho- and three retro-nasal attributes. Three taste and three mouthfeel attributes were also rated. Only

Enology – Aroma Compounds Session—CONTINUED

two ortho-nasal attributes differed significantly (artificial fruit/confectionary and herbal). The high CS leaf addition was the only treatment significantly different from the control for artificial fruit/confectionary. Most leaf treatments reduced perception of herbal attributes and had a pronounced artificial fruit/confectionary attribute. Increased perception of artificial fruit coincided with increased concentrations of fruity esters and grassy alcohols only for the large CS leaf addition, which had both significant chemical and sensory changes. The sensory results show that the primary difference between the wines was based on the freeze-killed leaf addition. Secondarily, the sensory data shows that at low leaf dosages, there isn't enough difference between the different frozen leaf additions to worry that leaves from different varieties would have substantially different chemical and sensory outcomes.

Funding Support: Washington Wine Commission,

Ambient *Cannabis* Aroma in Vineyards and Potential Wine Taint

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Airborne wine taint occurs when undesirable flavors and aromas are introduced to wine through exogenous sources. While the occurrence of environmental smoke taint and eucalyptus terpene uptake have been identified, the impact and risk of other common outdoor odors have not been investigated extensively. With the increasing prevalence of *Cannabis sativa* farms in New York, neighboring vineyard owners are concerned about a possible "*Cannabis* taint" in finished wine. This study investigated the effect on wine aroma when flowering *C. sativa* plants were placed directly in a Traminette (*Vitis* ssp.) vineyard, with grapes harvested at varying proximities (0, 2.7, and 16.2 m) to the *Cannabis* plants. Based on the results, it is unlikely that flowering *C. sativa* plants affect final wine aroma: following targeted analysis using solid-phase microextraction-Arrow gas chromatography-mass spectrometry, vinified wines showed no significant differences in the abundance of common cannabis aroma compounds. This suggests that white wine produced from vineyard sites neighboring *C. sativa* farms are unlikely to suffer from uptake of presently identified aromatic hemp compounds. Further, we determined the aroma detection threshold of four of the most abundant odorous *C. sativa* compounds in white wine for the first time, which will assist winemakers with evaluating potential odor impacts in their wine.

Funding Support: Cornell AgriTech Director's Fund; Hatch Grant #2022-23-205

Viticulture – Precision Viticulture Session

Accurate and Rapid Monitoring of Grape Ripening in the Field Through On-the-Go NIR/SWIR Hyperspectral Mapping

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Different factors can affect berry composition, such as soil characteristics, water availability, or other environmental factors. Knowing the chemical and physical variability of grapes in a vineyard can help manage growing conditions, avoid over-ripening berries, and plan the harvest. A UTV was adapted especially for this study

**indicates corresponding author*

Viticulture – Precision Viticulture Session—CONTINUED

to help lift the canopy and expose fruits. One hyperspectral camera in the NIR/SWIR domains was mounted on the back with GPS systems and halogen lights for night imaging. With this system, a Merlot vineyard located in Madera, California was imaged four times during the growing season and grapes were sampled for analysis in the laboratory. About 650 samples were collected and georeferenced. The grape signal was extracted through semantic segmentation and separated into grape, leaves, and background. The grape composition was predicted using four models: random forest (RF), extra tree regressor (ETR), extreme gradient boosting (XGboost), and gradient boosting (GB). The model's performances were assessed using 10-fold cross-validation and an external test set collected in a different vineyard and growing season. Predicting grape composition using the reflectance spectrum exhibited promising results, with ETR having the best performance for the prediction of total soluble solids (R2 = 0.91; NRMSE = 7%), pH (R2 = 0.90; NRMSE = 6 %), titratable acidity (R2 = 0.85; NRMSE = 7.5 %), and total anthocyanins (R2 = 0.91; NRMSE = 7 %). The models were used to develop prediction maps to understand the spatial variability of grape composition attributes in the vineyard and ripening heterogeneity. This study successfully proposes a system to accurately and rapidly monitor grape ripening in the field based on on-the-go hyperspectral mapping.

Funding Support: American Vineyard Foundation, California State University - Agriculture Research Institute

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Accounting for Spatial Variability to Efficiently Monitor Grape Composition in California Vineyards

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Vineyard spatial variability, the inherent differences found across the physical spaces of vineyards, creates challenges for vineyard and winery management. Vineyard managers are tasked with producing high-quality grapes at the highest yield possible and wineries expect fruit delivered from these vineyards to be of uniform quality, despite differences in spatial variability. In recent years, variable-rate management has been widely discussed, but few options are available for commercial use. While these technologies develop, a more practical approach must be found for efficient management of grapes in vineyards and wineries. In 2022, six Cabernet Sauvignon vineyards in the Lodi area were selected for canopy characterization and fruit compositional analysis. During the 2023 season, 10 Sonoma County and 18 Lodi Cabernet Sauvignon vineyards were selected for maturity tracking and fruit compositional analysis. In both years, plots were distributed based on a targeted histogram analysis of early-season Sentinel-2 NDVI images. This targeted histogram analysis entailed selecting field plots in low-, medium-, and high-vigor categories, and identifying a single, three-pixel transect that best represented high, medium, and low histogram variability, otherwise known as the "Best Fit (BF)". Results in 2022 showed that fruit zone light interception and fruit composition differed among high, medium, and low vigor zones, with the BF representing variability in most vineyards. The 2023 results confirmed that the BF could be used to monitor vineyard maturity in commercial vineyards. Variability caused by pest or disease pressure, topography, or vineyard management decisions created some limitations for the BF method and must be considered when deploying image-based decision platforms.

Funding Support: NA

Viticulture – Precision Viticulture Session—CONTINUED

Relationships among Vine Nutrient Status, Canopy Size, Vine Physiology, Yield, and Grape Aroma within Vineyards

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Several grape cultivars are characterized by a rich assortment of volatile terpenoids (mono- and sesqui-), a class of secondary metabolites that affect grape and wine aroma. Intra-vineyard factors that affect volatile terpenoids are not well known, despite potentially affecting the quality of grapes and wines. This study aimed to assess the relationships between grape volatile terpenoids and other vine parameters within vineyards. Two commercial vineyards (Riesling and Gewürztraminer) in the Okanagan Valley (British Columbia, Canada) were considered. To assess intra-vineyard variation, 40 and 48 plots distributed within the Riesling and Gewürztraminer vineyards, respectively, were selected. Three random vines per plot were used for all measurements. These included plant water status (stem water potential), leaf area, and leaf gas exchange (CO_2 assimilation, transpiration, stomatal conductance), measured at bloom and veraison. At the same stages, normalized difference vegetative index (NDVI) and normalized difference red edge index (NDRE), calculated from remote sensing-based multispectral drone images and ground-based measurements, were used to estimate vine vigor. Vine nutrient status was determined by petiole analysis at veraison. Yield, berry technological quality (total soluble solids, pH, and titratable acidity), and volatile terpenoids were determined at harvest. The content of grape volatile terpenoids correlated positively with leaf area, NDVI, NDRE, and yield in Riesling, and with petiole nitrogen content, NDVI, NDRE, and yield in Gewürztraminer. Our study confirms that remote sensing-based NDVI and NDRE correlate positively with petiole nitrogen content, leaf area, and yield, and indicates that these parameters could predict variation in grape volatile terpenoids within vineyards. Our results also suggest that, in the Okanagan Valley, promoting vine vigor can improve grape aroma, and remote sensing-based vegetative indices could serve as a cost-efficient and time-saving approach to estimate vine vigor within a vineyard.

Funding Support: MITACS/ Investment Agriculture Foundation

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Enology – Fermentation Management Session

A Current Evaluation of Wine Fermentation Models

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The field of wine fermentation modeling can be traced back to the late 1970's, when alcoholic fermentation models were first extended to address initial sugar concentrations of 200 to 240 g/L, ethanol concentrations of 100 to 110 g/L, a mixture of glucose and fructose and the decline in cell viability. Today, wine fermentation models, in combination with sensors, are being used to provide early diagnosis and prediction of abnormal fermentations at commercial wineries. In this work, multiple wine fermentation models were compared for their mathematical descriptions of cell growth, substrate consumption, product formation, temperature variation, cell death and the calculation of density from solution composition and solute properties. The models were also evaluated on a red and white commercial fermentation data set across a wide range of winemaking conditions. Only one model appeared suitable for application to commercial fermentations. The limitations of current models and suggestions for areas of advancement in the field are presented.

Funding Support: T.J. Rodgers University Fellowship in Electrical and Computer Engineering; Rossi Endowment in Viticulture & Enology

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Effect of Cap Management Frequency on the Phenolic, Chromatic, and Sensory Composition of Cabernet Sauvignon Wines

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Cabernet Sauvignon (clone 7) from the Paso Robles AVA was processed with a contrasting array of punch-down frequencies, ranging from zero to four punch-downs/day, over two vintages (2019 and 2020). In one vintage, fruit was harvested at two contrasting maturity levels. The only variable was the frequency of punch-down, with temperature and other variables kept constant within treatments. Wines were followed for up to three years of bottle aging for basic and phenolic chemistry, and the late-harvest wines of 2020 were subjected to sensory analysis. There were no effects of punch-down frequency on alcohol, pH, titratable acidity, or lactic acid. This is likely because all the wines, irrespective of punch-down frequency, were submitted to very similar temperature regimes. There was a rather unremarkable effect of punch-down frequency on the basic phenolic composition of the wines, including anthocyanins, tannins, and polymeric pigments. These results counter empirical winemaking observations, whereby more frequent physical mixing should lead to higher phenolic extraction. It is possible that more frequent mixing may lead to phenolic losses due to chemical and/or enzymatic oxidations. Such losses may be minimized under conditions of no mixing, akin to wines made with no cap management. In fermentors of relatively small volume (60 L) and at high fermentation temperature, convection movements may allow for sufficient phenolic extraction. This may extend to fermenters up to 1.5 tons; however, reduction aromas may concomitantly appear as well under such conditions. Sensory results indicated that all four wines, irrespective of punch-down frequency, were perceived as drying. While other subqualities of astringency changed little as a function of the treatments, the perception of bitterness increased concomitantly with the frequency of punch-downs, with 3 punch-down wines showing the greatest bitterness perception.

Funding Support: Agricultural Research Institute (ARI)

Enology – Fermentation Management Session—CONTINUED

Effects of Including Different Forms of Grape Stems on Chemical Parameters of Red Wines

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There is increased interest in using grape stems in red winemaking, but their actual impact on wine quality is poorly understood. Grape stems are either not harvested by mechanical harvest, or are discarded after manual harvest and the destemming process. However, grapes stems are rich in phenolic compounds, which makes them a potential good source of tannins to improve red wine quality. In 2023, two cold-hardy grape cultivars, Marquette and Frontenac, were crushed, destemmed, and processed with different stem additions. Stems were collected and either kept fresh (FS), oven-dried (DS), or oven-dried and ground (DGS) prior to being added to the musts. Those conditions were compared to a control treatment to which no stem was added. Red wines were made in triplicate for each condition following a standard red winemaking procedure, using yeast strain ICV D254 and co-inoculated with VP41 malolactic bacteria. Basic chemistry, color parameters, and phenolic compounds of the resulting red wines were analyzed by UV-vis spectrophotometer and high-performance liqud chromatography diode array detection at bottling. The pH was higher in Frontenac than in Marquette wines, but pH and titratable acidity were not affected by the treatments. The color intensity was greatest in control Frontenac wines and least in FS Frontenac wines. This could be explained by the ability of anthocyanins to bind with cell wall material of fresh stems. However, no effect of stem inclusion on color intensity was observed in Marquette wines, which may be due to their different anthocyanins profile. The FS and DS Marquette and Frontenac wines contained significantly more tannins than the control wines. Surprisingly, the concentrations of phenolics and tannins were not improved by DGS, suggesting a lower impact on the surface area-to-juice ratio on tannin extraction.

Funding Support: This project was supported by the U.S Department of Agriculture's (USDA) Agricultural Marketing Service through grant 23SCBPIA1187.

Isothermal Titration Calorimetry to Describe Polyphenol-Macromolecule Interactions

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This study builds on our previous findings that grape flesh-derived macromolecules, mainly polysaccharides and proteins, significantly affect retention of tannins and anthocyanins during winemaking. We specifically examined how anthocyanins in mono- and diglucoside forms influence these interactions. Employing isothermal titration calorimetry (ITC), we evaluated the binding affinity and thermodynamic properties between poly(L-proline) (PLP, at 0.7 mM) and pectins (at 30 mM galacturonic acid eq.) with commercial grape tannin (BioTan) and procyanidin C1 (both at 3 mM epicatechin eq.). To assess whether anthocyanins alter the binding interaction of tannins with these macromolecules, we conducted a pre-incubation of the macromolecules with malvidin-3-glucoside (M3G) and malvidin-3,5-diglucoside (M35DG), each at a concentration of 3 mM prior to titrating with tannins.

Tannins showed a strong affinity for PLP, while no interaction was observed with pectin, even at greater concentrations. Tannins did not interact with pure anthocyanin solutions under the experimental conditions. Interestingly, pre-

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Enology – Fermentation Management Session—CONTINUED

incubation of PLP with M35DG reduced the binding affinity of BioTan to PLP, while M3G did not significantly alter this affinity. The nature of the tannin-PLP interaction, whether enthalpy- or entropy-driven, varied based on the type of tannin.

The different effects of malvidin mono- and diglucoside on the strength of interaction between tannins and proteins help clarify the effects of different grape varieties on the sensory attributes and color stability of red wine. This could be used to manage the texture and mouthfeel of wine and to adjust winemaking processes such as fining, filtration, or blending. Because we observed haze formation during the ITC experiments, future research will focus on investigating the aggregation patterns between polyphenols and macromolecules using dynamic light scattering.

Funding Support: No funding source.

Viticulture – Pest and Disease Session

Assessing Different *Botrytis cinerea* Strains using Molecular Biological Methods

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The fungal pathogen *Botrytis cinerea* has a tremendous impact on many crops, including winegrapes. It causes specific off-flavors, brownish color, and poor filtrability in must and wine, and can lead to total loss of harvest. Climate change and development of strains resistant to fungicides make it even more challenging to control the disease. To adapt to those problems, it is important to find and develop new methods to detect *Botrytis* and differentiate strains. Some of these methods are strain differentiation, classification by simple sequence repeats (SSRs), and early detection of the fungus by qPCR. In this ongoing study, strains from different regions, years, and grape varieties were analyzed using SSR markers and evaluated using either agarose gel electrophoresis or capillary sequencer via PCR. Furthermore, a sensitive qPCR method was refined to achieve early detection of the pathogen. In addition, cross-contamination with other grape pathogens, *Penicillium expansum, Trichothecium roseum, and Cladosporium spp*., was tested to exclude false quantification of the biomass. The qPCR method was also tested with different *B. cinerea* strains, to test for potential quantification differences between strains. The results demonstrate promising ways to distinguish between strains using both agarose gel electrophoresis and capillary sequencing, as well as to detect an infection with qPCR before it becomes visible on grapes. Cross contamination could be excluded with the tested pathogens. The different *Botrytis* strains tested in qPCR had no significant affect on quantification of the biomass. The results can be used to further understand and analyze different *B. cinerea* strain characteristics, such as laccase activity, regional, or annual effects. The early detection method can be used to better prepare growers for an impending infection so that targeted efforts can be made.

Funding Support: Forschungskreis der Ernährungsindustrie E.V. (FEI)

Viticulture – Pest and Disease Session—CONTINUED

How to Block the Spread of Pierce's Disease: Gene-Editing of the Pierce's Disease Vector, the Glassy-Winged Sharpshooter

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Pierce's disease is a serious disease of California grapevines caused by a pathogenic bacterium, *Xylella fastidiosa*, which is transmitted by a xylem-feeding insect, *Homalodisca vitripennis* (the glassy-winged sharpshooter, GWSS). Current methods to control this invasive pest are expensive, relying on insecticides, quarantine, and eradication. By blocking the ability of GWSS to transmit *X. fastidiosa*, we can control the spread of Pierce's disease in a sustainable manner. Our control strategy is feasible due to the powers of clustered, regularly-interspaced, short palindromic repeats (CRISPR)-based gene-editing technologies. CRISPR-based technologies will allow us to generate GWSS strains that are unable to acquire *X. fastidiosa* from infected plants or transmit *X. fastidiosa* to healthy grapevines. We have developed these technologies for GWSS. We have used CRISPR technologies to knock out a gene or to insert a gene into the GWSS genome. As proof of principle, we generated the first genetic mutants of GWSS using two eye pigmentation genes, white and cinnabar. We obtained mutants at high frequency, these mutant strains are robust, and we have maintained the strains in our laboratory for over 15 generations. We confirmed that the white and cinnabar mutations are specific to the target sites, which is critical for genetic control strategies. We have also demonstrated that we can integrate DNA fragments and genes into specific target sites in the white and cinnabar genes at high frequencies. This technology has allowed us to establish a platform for rapid screening of gene regulatory sequences in GWSS. We still need tissue-specific gene regulators for our genetic control strategy. With the gene-editing technology firmly established in GWSS, we are now identifying genes that can be used to block GWWS' ability to acquire or transmit *X. fastidiosa*. Our goal is to make a *Xylella*-proof GWSS and end the threat of Pierces' disease in California.

Funding Support: Our research was funded by the Pierce's Disease/GWSS Board, California Department of Agriculture and the Animal and Plant Health Inspection Service of the United States Department of Agriculture.

THURSDAY ORAL ABSTRACTS

Fungicide Resistance in Powdery and Downy Mildew in Australian Vineyards

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Grapevine powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) are significant diseases in Australia and worldwide. Fungicides are the key to managing these diseases. However, frequent use can lead to fungicide resistance. Samples were collected from five states in Australia from 2017 to 2023 and phenotyping and genotyping were used to investigate resistance status. Phenotyping was conducted for six and four fungicide groups for powdery and downy mildew, respectively. Both pathogens had varying levels of reduced sensitivity to most fungicide groups tested, with resistance confirmed for fungicides from quinone outside inhibitors (QoI, group 11) and proquinazid (group 13) for *E. necator* and phenylamide (group 4) and QoI for *P. viticola*. Two mutations, G143A and Y136F, were identified in *E. necator* populations. There were strong relationships between reduced sensitivity to QoIs and the presence of the G143A mutation in the CYTB gene, but not

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Viticulture – Pest and Disease Session—CONTINUED

between reduced sensitivity to demethylation inhibitor (DMI) and Y136F mutation in the CYP51 gene. The mutant H242R/Y, associated with resistance to succinate dehydrogenase inhibitors (SDHI, group 7), was not detected, but reduced sensitivity was recorded for this group. G143A was also detected in *P. viticola* isolates, with a strong relationship between phenotype and genotype for QoI. Techniques are being improved to increase the monitoring capacity for fungicide resistance using rotorod spore traps and other sample collection methods to detect mutations linked with resistance. High-throughput laboratory and in-field qPCR methods are being developed and validated, with high-throughput sequencing to evaluate their capability to rapidly and cost-effectively identify fungicide resistance mutants.

Funding Support: Wine Australia and CRC-SAAFE

Field Application of Biofungicides to Control Powdery Mildew and Botrytis Bunch Rot on California Winegrapes

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Grapevine powdery mildew caused by *Erysiphe necator* and Botrytis bunch rot, caused by *Botrytis cinerea*, are the two of the most important fungal diseases for California grape production. Growers are increasingly interested in using biofungicides because of tightening state regulations and the development of fungicide resistance in fungal populations. We initiated biofungicide trials in 2023 in two CA regions: CA Central Coast (San Luis Obispo), using Chardonnay and Pinot noir, and the San Joaquin Valley (Fresno), using Chardonnay and Carignan. At each location, two blocks were selected per variety. Weather stations were installed in the vineyards before budbreak to monitor the real-time powdery mildew risk index. Fourteen treatments included three biofungicides (*Bacillus subtilis*, *Streptomyces lydicus*, and extract of *Reynoutria sachalinensis*) applied at different intervals (weekly, biweekly, and mildew risk index-based), biofungicides rotating with synthetics, synthetic standard only, and an untreated control. Treatments started at bloom. Disease incidence and severity were rated biweekly, approximately one week prior to veraison, and yield and berry chemistry were measured at harvest. Biofungicide by itself did not show a better efficacy than synthetic standard on disease incidence or severity. However, the rotation of bio-fungicide and synthetic showed similar efficacy against disease severity as synthetic standard alone on both varieties at each location. No significant difference in yield or berry chemistry was found between biofungicide and synthetic treatments. The ongoing project will be repeated in 2024.

Funding Support: CDFA-Specialty Crop Block Grant Program and California State University Agricultural Research Initiatives

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Enology and Viticulture Research Report Posters

Evaluation of Alternative Varieties in the Willamette Valley AVA using the Average Growing Season Temperature Index

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This analysis is a follow-up to a recent study that examined the ripening potential of Pinot noir in Oregon's Willamette Valley (WV) American Viticultural Area (AVA). The study involved computations of the growing season average temperature (GST) viticulture climate classification index on a mean decadal basis from the 1950s to the 2090s using coupled model intercomparison project phase 5 (CMIP5) RCP4.5 and RCP8.5 projections of minimum and maximum daily surface air temperature. It considered the complete archive of the 32 CMIP5 daily localized constructed analogs downscaled historic data sets and their observational data that were used for downscaling and bias corrections to develop a weighted ensemble that optimized calculation of the GST index throughout the WV AVA. Using the GST index value range from 14.0 to 16.0°C for optimum suitability of Pinot noir (*Vitis vinifera* L.), the study results showed a progressive trend of decreasing area to support Pinot noir in the WV AVA for each RCP-emission scenario, which was more pronounced for the RCP8.5 scenario projections. This study applies the temperature-based GST bioclimatic index calculations that were developed for the WV AVA and the individual optimal GST index value ranges proposed by Greg Jones in 2006 for 20 other *Vitis vinifera* L. varieties, to evaluate alternative varieties that may be more suitable for ripening in the WV AVA due to climate change.

Funding Support: NA

Assessing Long-Term Effects of Regenerative Management Practices on Vineyard Soil Health

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Vineyards are often developed on slopes or marginal lands, which results in soils that are particularly susceptible to erosion and degradation. To mitigate these impacts, regenerative management methods—including the use of cover crops, no-till, compost application, and livestock integration— can be used to improve soil health and increase soil organic matter. These practices can improve crop productivity and quality, water infiltration, nutrient availability, and carbon sequestration within soils. Because building soil health is a slow process, there is little knowledge regarding the effects of long-term use of regenerative management practices on vineyard soil health.

To improve soil health and create more resilient agricultural systems, this project aims to assess the effects of long-term management practices on vineyard soil health across an edaphoclimatic gradient in California. Soils were collected from 87 different vineyard blocks across CA, specifically targeting vineyard blocks where one or several regenerative practices have been adopted for five or more years. Following recommendations from the Soil Health Institute, all samples were analyzed for a minimum suite of soil health indicators, which included soil organic carbon concentration, carbon mineralization potential, and aggregate stability. For each vineyard block, growers completed a qualitative survey on the management history and the performance of the vineyard, including yield, crop quality, water, nutrient, and pest management. Our preliminary results show that finer-textured soils had

**indicates corresponding author*

Enology and Viticulture Research Report Posters—CONTINUED

more total soil carbon concentrations than coarse-textured soils in the vine and alley row. Total soil carbon concentrations in the vine and alley rows increased significantly with the number of years livestock integration had been adopted. Additionally, the years of livestock integration and adoption of no-till increased aggregate stability indices within the vine row. Texture class and location were more important in predicting total carbon and aggregate stability indices than the duration of practice adoption.

Funding Support: Foundation for Food and Agricultural Research, and CDFA

Influence of Vineyard Planting on Soil Physical and Hydraulic Properties

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Vineyard soils are most disturbed during vineyard establishment, including pre-plant soil preparation, planting, and trellis installation. This process grossly alters soil physical and hydraulic properties in a repeating pattern across newly planted vineyard blocks. To study the influence of mechanical vineyard planting on soil hydraulic and physical properties, a suite of analyses was conducted on soils and soil cores taken from the vine (3) and tractor (3) rows of a newly mechanically-planted vineyard and an adjacent property of mixed uses, including as a vineyard, that has not been disturbed in at least five years. Soil bulk density was 30% greater in the tractor row and 20% greater in the no-till field compared to the vine row of the recently planted vineyard. Soil saturated hydraulic conductivity (Ksat) was significantly increased (100x) in the vine row compared to the tractor row. No-till soils had a Ksat ~10x greater than the tractor row and 10x less than the vine row, representing an almost optimal infiltration rate of ~20 cm/hr. Mean porosity measured by x-ray computed tomography was greatest in vine rows and least in the tractor rows. No-till soil showed not just extensive root colonization, but also extensive exploration by earthworms, contributing to the macroporosity and thus contributing significantly to the observed Ksat in the no-till soil. These results could guide vineyard planting practices to minimize soil disturbance and highlight the synergy between cover crops and soil fauna in improving important soil health properties like capturing water.

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Using Passive Samplers to Measure Volatile Phenols in Smoke to Correlate to Concentrations in Grapes and Wines

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Smoke generated from wildfires can detrimentally affect grape and wine composition through increased concentrations of volatile phenols (VPs) and their glycoconjugates (bound), a phenomenon termed “smoke taint”. This study evaluated passive samplers as a method for monitoring the levels of VPs in ambient wildfire smoke that are known to contribute to smoke taint in wines. Cabernet Sauvignon grapes and passive samplers were exposed to smoke under controlled conditions to evaluate the efficacy

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of passive samplers across various smoke intensities. Smoke was generated from oak chips using a custom-built smoking chamber that consisted of: an infrared heating burner system, a polycarbonate chamber internally lined with Teflon sheets, and a ventilation system to operate the system under negative pressure and eliminate emissions. Grapes and samplers were exposed to four different smoke levels: control exposure (CE, no smoke), low exposure (LE, 0.5 hrs, ~20 g oak wood), medium exposure (ME, two hrs, ~60 g oak wood), and high exposure (HE, four hrs, ~180 g oak wood). Afterward, triplicate microfermentations (0.5 L) were performed per treatment. Passive samplers were analyzed using thermal desorption (TD) coupled to gas chromatography-mass spectrometry (GC-MS). Grapes and wines were analyzed for free and bound VPs using GC-MS/MS (free) and LC-MS/MS (bound). Free and bound VPs in grapes and wines correlated positively with VP levels in passive samplers. Linear regression analysis of free VPs indicated a good linear fit between passive samplers and grape samples for all compounds, with R^2 values between 0.9999 and 0.9985 for guaiacol, methyl guaiacol, 4-ethyl guaiacol, and *o*-, *p*- and *m*-cresol. This study was the first step to determine the efficacy of using passive samplers as an early warning system in vineyards. Future experiments will correlate passive sampler VP levels to grape and wine composition across several smoke intensities, exposure durations, and repeat exposures to build a predictive model.

Funding Support: USDA-ARS funded research

Heard it Through the Grapevine: Assessing the Spatial Variability of Hydrometeorological Controls in Canadian Vineyards

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Canada's wine industry contributes over $11 billion toward the national economy, of which $5.5 billion is generated by Ontario wineries. The cultivation of high-quality grapes to produce economically valuable wines does not come without challenges to growers and winemakers alike. Spatial variability in microclimate and soil conditions have been a long-standing challenge to growers since the start of grapevine cultivation. With the added pressures of adapting to climate change, vineyards will require special management adaptations to achieve high-quality berry yields and wines. The role of spatial variability on grape quality is especially important within the three principal growing regions of Ontario, Canada. Here, climate is shifting toward longer and warmer growing seasons, with variable precipitation patterns that present additional threats to growers, in addition to those that already exist due to vineyard spatial variability. This research used a combination of methods aimed at assessing hydrometeorological components, site-wide and between different blocks and rows and among individual grapevines, to best understand spatial differences across two vineyards in the Niagara growing region. VineTalkers, an innovative new tool for collecting biophysical data across large heterogeneous landscapes from individual plants, collected data on water transport, leaf spectrometry, below-canopy radiation, and various microclimatic measurements. Preliminary results showed homogeneity across blocks for subcanopy air and soil temperatures, while variability was present for soil moisture and sap flow (transpiration) conditions. This data, paired with meteorological and eddy covariance tower findings, presents an opportunity for growers to understand the current state of their vineyards and the spatial variability across their crop, allowing them to make specific, non-uniform management decisions to enhance grape yield and quality each growing season.

Funding Support: Global Water Futures, Ontario Grape and Wine Research Inc.

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Sunscreen for Grapevines: Kaolin Clay Use in Pacific Northwest Vineyards

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Short-term climate change effects like extreme heat threaten grape production in the Pacific Northwest. Extreme temperatures inhibit photosynthesis, reduce yield, delay ripening, and promote fruit sunburn. Kaolin clay is used in other crops to reduce heat stress and sunburn. Additionally, other labeled (insect suppression) and experimental (wildfire smoke exposure) uses distinguish kaolin as a viticultural tool to mitigate climate change-related risks.

Multiple field experiments were established in 2022 and 2023 in commercial vineyards near Milton-Freewater, Oregon to optimize kaolin application (i.e., rate, timing, adjuvant use), observe fruit sunburn effects, and measure effects on leafhopper populations. In one block of *Vitis vinifera* L. cv. Syrah, kaolin was applied between fruit set and harvest at varying rates (0, 11, 22, or 45 kg/ha), at different times (pre- and postveraison), and with commercial adjuvants. The effect on sunburn was tested with fruit zone leafing on the afternoon side of Syrah and Tempranillo vines with label rate kaolin application to half of clusters prior to a postveraison heat event (three days above 41°C). At a second site, kaolin was applied to organic Cabernet Sauvignon and populations of *Erythroneura elegantula* adults and nymphs were monitored from bloom until harvest.

In 2022, kaolin at 45 kg/ha improved stomatal conductance above Ψ_{leaf} of -1.4 mPa, increased fruit yield by 5%, and slightly improved anthocyanin concentration. In 2023, tannin and anthocyanin concentrations increased with application rate and were greater with preveraison application. Visual assessment indicated that all adjuvants dramatically improved dispersion and deposition of kaolin on berries. Application prior to a heat event reduced the impact of sunburn on marketable yield and quality; treated clusters had 52 to 85% fewer shriveled berries and up to 25% higher anthocyanin concentration. Kaolin application reduced the peak population of second generation *E. elegantula* nymphs by 54%.

Funding Support: Agricultural Research Foundation, Oregon Wine Research Institute

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Smoke Volatile Phenol Diffusion Capabilities through Cellulose Nanofiber-Based Films

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The growing concern of wildfire smoke affecting winegrapes around the world has triggered an immediate need to develop mitigation techniques. Smoke exposure can lead to negative qualities in finished wine. Current remediation strategies have not been effective in removing smoke taint in wine. One promising technique is spraying winegrapes with cellulose nanofiber-based films (CNF) prior to a smoke event. Previous research has shown that when these films were applied in the field before smoke exposure, the resulting grapes had lower concentrations of guaiacol, 4-methylguaiacol, syringol, and 4-methylsyringol when compared to grapes with

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no films. This study aims to analyze the diffusion capabilities of the volatile phenols through the films. Four different film types were analyzed. To a base of CNF, different quantities of chitosan and/or β-cyclodextrin were added. Each film was placed inside a custom-made polytetrafluoroethylene two-piece chamber. The bottom piece of the chamber contained a volatile phenol, and the film was placed in the middle of the chamber. Headspace samples were removed from the top of the chamber. Samples were analyzed using gas chromatography-mass spectrometry. Differences between diffusion permeability of different phenols were found based on film type. Modifications can be made to current films to prevent a wider range of smoke compounds from being absorbed by winegrapes. Results from this study are critical in developing coatings that can prevent smoke compounds from entering grapes, should a wildfire occur.

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HiRes Vineyard Nutrition Project Seeks to Develop New Tools and Refine Nutrient Thresholds

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Vineyard nutrient management is essential to maintain vine health, productivity, and fruit quality targets. However, conventional vine tissue methods are labor intensive, costly, lack standardization, and lack the ability to describe nutrient status spatially across vineyards. Our interdisciplinary team is refining tissue sampling techniques and creating decision-support remote-sensing tools for real-time assessment of vineyard nutrient status. The project has four objectives: 1) develop non-destructive tools to measure grapevine nutrient status, 2) determine the efficiency and suitability of precision vineyard nutrient management, 3) define grapevine nutrient thresholds based on environment and production market, and 4) estimate the economic impact and feasibility of nutrient management decisions and extend knowledge. The project is generating vineyard nutrient prediction maps by employing hyperspectral sensors calibrated against vine tissue nutrient data. Field trials involving nitrogen, potassium, and magnesium supplementation in wine, table, raisin, and juice grape varieties were conducted according to local nutrient-limitation conditions in WA, OR, CA, NY, and VA. To address improved season-long sampling, tissues were collected and analyzed at growth stages spanning from dormancy to leaf fall. To share project updates with industry and peers, the team provided field events, podcasts, and train-the-trainer programs. By integrating new technologies with vineyard nutrient management practices, this project aims to provide stakeholders with new options for vineyard nutrient monitoring that will improve decision-making within the grape production sector.

Funding Support: USDA National Institute of Food and Agriculture-Specialty Crop Research Initiative Coordinated Agricultural Projects (CAP) grant project award number: 2020-51181-32159.

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Impact of Grapevine Red Blotch Virus Infection on Grape Skin Cell Wall Composition over Two Growing Seasons

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Grapevine red blotch virus (GRBV), a member of the Geminiviridae family and vectored by the three-corned alfalfa treehopper *Spissistilus festinus*, causes delayed grape ripening and decreased wine phenolic content. However, wine phenolic content does not correlate linearly with concentrations in grape skin cell walls (GSCW). Phenolics are stored in GSCW vacuoles with extraction into macerating juice affected by the hemicellulose, pectin, and structural protein content of cell walls. GRBV's effect on GSCW was studied using virus-infected, GRBV(+), and noninfected, GRBV(-) vines from a commercial vineyard in Paso Robles, CA in 2021 and 2022. Biochemical analysis involved GSCW isolations as alcohol-insoluble residue, total protein content (TPC) using the Coomassie blue protein assay, and pectin concentration measured as uronic acid. TPC was lower in GRBV(+) than in GRBV(-) in 2021 and 2022 grapes at harvest and in postveraison GRBV(+) grapes in 2021. In 2022, there was less TPC in GRBV(+) and GRBV(-) grapes than in 2021. TPC decline in 2022 can be attributed to increased heat degradation or suspension of total protein production due to environmental factors such as more growing degree days and lower annual precipitation. Further investigation will uncover the relationship of TPC with pathogenesis-related protein production during infection. GRBV(+) GSCW exhibited greater pectin in both seasons, possibly due to a ripening delay with downregulated pectin enzyme activity or upregulated pectin synthesis, since concentrations in GRBV(+) grapes at preveraison, postveraison, and harvest in both vintages were greater. Pectin concentration has been previously proven to be inversely correlated with phenolic extractability due to firmness of the GSCW. Future steps involve using gas chromatography coupled with mass spectrometry to assess the effect of GRBV on GSCW monosaccharide linkages.

Understanding the viral effect on GSCW and its correlation with phenolic extractability will help determine mitigation strategies for the winemaking industry.

Funding Support: CDFA-PD/GWSS Board, Jastro-Shields Research Award, American Society of Enology and Viticulture Traditional Scholarship, NIFA Specialty Crops Research Initiative

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Exploring Berry Astringency in Table Grapes

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Scarlet Royal, a mid-season ripening table grape, is a popular red grape variety in California. However, its berries develop an undesirable astringent taste under certain conditions. A series of trials have been conducted to comprehend the underlying mechanism and control of astringency development. First, we used total phenolic analysis to determine the components responsible for the astringent taste. Second, we identified the pathway involved in this process. By comparing Scarlet Royal berries with non-astringent varieties, we determined the threshold of tannin levels that cause the berry's astringent taste. Our data showed that berry astringency is correlated with high petiole nitrogen levels and can be managed through N fertilization. Additionally, our data showed that a lower cluster number per vine

could contribute to the induction of berry astringency. Further, berry astringency is associated with late harvest dates after the middle of September; however, it is not associated with high sugar content, as some people believe. It was associated with a lower level of anthocyanin in the berries at harvest. Table grapegrowers could control the induction of berry astringency in Scarlet Royal table grape by monitoring and maintaining a reasonable N level during flowering and berry development, and by not reducing the cluster number to 20 clusters per vine.

Funding Support: California Table Grape Commission

Could Foliar-Applied Prohexadione Calcium and Phenylalanine Affect the Phenolic Content of Marquette?

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Cold-hardy hybrid grape cultivars have less tannin than *Vitis vinifera* cultivars, which leads to low-quality, unbalanced red wines. Foliar sprays of phenylalanine (Phe; 100 mg/L) and prohexadione calcium (ProCa; 50 mg/L) were applied separately and in combination (ProCa + Phe) to Marquette grown in Iowa throughout the 2022 and 2023 growing seasons. ProCa was applied to change the plant cell wall structure and modify tannin extractability. Phe is a precursor of flavanoid biosynthesis. Treatments were assigned following a split-plot design to three-vine panels with six replicates. Samples from treated vines were compared to untreated control samples using an additive effects linear regression model with random intercepts. Basic chemical properties, tannin content (high-performance liquid chromatography-diode array detection), and iron-reactive phenolic (UV-vis spectrophotometry) content of juice, wine, and skin and seed extracts were analyzed at five phenological time points. In 2022, the ProCa treatment had lower titratable acidity at three weeks post-fruit set, but Brix, pH, hue, and color intensity were unaffected at any time point. At the mid-ripening time point, Phe showed a positive effect on juice tannin concentration. However, the model showed that when Phe and ProCa + Phe treatments were applied, skin tannin and iron-reactive phenolic contents were negatively affected. Seed phenolic content was not affected by foliar-applied Phe or ProCa. Overall, limited effects on tannin and IRP contents were observed in different grape tissues, which may be explained by the low doses of Phe and ProCa used in this study. However, analysis of 2023 data is ongoing to investigate the effectiveness of Phe and ProCa foliar sprays at managing phenolic content in Marquette grapes over two growing seasons.

Funding Support: No external funding.

Exploring Auxin Responsive Factor 4's Influence on Timing of Ripening Initiation Using Molecular Breeding

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Genetic manipulation is a major tool in determining gene function and its relationship with traits of interest in plants. For any crop, it requires a tractable genetic transformation system and a reliable expression system to turn on or switch off expression of targeted genes. We implemented the microvine system for *Agrobacterium tumefaciens*-mediated transformation and a plant gene switch system to control the transgene expression by supplying transgenic greenhouse

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plants (through root-drenching) a chemical inducer, methoxyfenozide. As proof of principle, we conducted gain and loss of function studies on Auxin Responsive Factor 4, a transcription factor that may contribute to the timing of ripening initiation in grape berries. We developed three types of constructs. In construct 1, we aimed to induce over-expression of the native endogenous ARF4. In construct 2, we generated transgenic lines containing a synthetic ARF4 resistant to miRNA-mediated gene silencing (miRARF4). In construct 3, we created ARF4-silencing lines using artificial miRNA technology. To pinpoint plants with the most response to the chemical inducer, eight plants from each construct were grown to maturity and induced over a month with leaf collections at select points. Six collection times were chosen to comprehend both the short-term responses (days 0, 3, 7, and 10) and the reversibility of the system (days 14 and 31). The data extracted using qPCR allowed us to characterize the time period of greatest induced transgene expression. Expression levels of the most responsive plants were eight-fold in the native construct, 100-fold in miRARF4, and 1000-fold increase in the ARF4 silencing line. Ongoing experiments quantitatively compare expression levels in berry samples to verify the inducibility dynamics in the fruit. Once confirmed, the study intends to focus on comparing ARF4's targetome among the three constructs by using genome-wide transcriptomic analyses.

Funding Support: Oregon Wine Board, Erath/Family Foundation, Oregon Wine Research Institute

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Identifying Protein-Protein Interactions in Red Blotch Infected Vines

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Grapevine red blotch virus (GRBV) poses a significant threat to winegrape production. Host factors critical for red blotch infection remain largely unknown in grapevines, impeding attempts to use CRISPR technology to generate red blotch-resistant grapevines. Our project seeks to identify these host factors. Our two main objectives are to identify the host factors using the yeast two-hybrid (Y2H) system and to validate their interaction with viral proteins *in planta*. For the Y2H experiment, we will conduct a comprehensive genome-wide screen using GRBV replication-associated proteins (C1, C2, C3, and C1-2) as baits against a comprehensive grapevine cDNA library from GRBV-infected plants as preys. Based on the strength of the interactions and their biological relevance from previous studies, we will select the top two most promising interactors for each bait (C1, C2, C3, C1+2) to conduct bimolecular fluorescence complementary assays from isolated grapevine protoplasts. All four baits have been successfully cloned into yeast, initiating the screening process. A prey library using coding-gene related RNA is currently being constructed. The next step will be to carry out mating of the bait and prey, as well as isolation of mated yeast. Further screenings on a series of selective media will identify high-confidence interactors, followed by Sanger sequencing to identify their biological function. To get ahead of the work on objective 2, we have optimized a protocol to isolate protoplasts from tissue cultured cells of microvines. If we reach our goals, we will lay the groundwork for further studies to produce CRISPR-engineered, GRBV-resistant grapevines.

Funding Support: California Department of Food and Agriculture

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A Two-Year study of 12 Sustained and Regulated Deficit Irrigation Schedules for Cabernet Sauvignon in Central California

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Amidst escalating drought conditions in the San Joaquin Valley, where grapegrowers face an urgent need for effective water management strategies, this study contributes valuable insights for sustainable viticulture practices. Recognizing the pressing importance of optimizing water use in vineyards, particularly in the context of the West San Joaquin Valley, this research aims to unravel the physiological responses of grapevines under varying intensities and timings of sustained and regulated deficit irrigation.

Focused on a singular commercial Cabernet-Sauvignon 1103 Paulsen vineyard, this study explored the impact of irrigation treatments administered during 2022 and 2023 seasons. Employing a semi-autonomous irrigation approach, actual water quantities were recorded using flow meters. The study incorporates sustained deficit irrigation (SDI) schedules with varying percentages (40, 60, 80 and 100% of ET_c) and regulated deficit irrigation (RDI) strategies in both preveraison and postveraison periods (e.g., 100/40, 80/60, 60/100).

Stem water potential and gas exchange were measured from June to harvest, grape composition from veraison to harvest and yield components were also assessed at harvest time. Results show that while the 40% SDI significantly underperformed 100% ET_c, it is possible to obtain similar or better results while saving water with RDI and SDI treatments with lower ET_c reductions.

This research determined the primary effects of these irrigation regimes on plant water status, gas exchange, berry composition, and yield components. The findings aim to provide a nuanced understanding that can inform advanced developments in water management strategies, offering practical insights for grapegrowers navigating the challenges of water scarcity in the San Joaquin Valley.

Funding Support: American Vineyard Foundation, California State University - Agriculture Research Institute

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Enhancing Water Use Efficiency and Berry Ripening in Cabernet Sauvignon: Effects of Irrigation Regimes and Biostimulants

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Water availability is a key factor for agriculture, as rising temperatures increase plants' water demand, while water resources become increasingly limited.

Optimizing water use efficiency is essential to enhance vine tolerance to heat stress and ensure high productivity. This study examines the effects of four distinct irrigation regimes: vines irrigated at 50, 75, and 100% of estimated crop evapotranspiration (ET_c), alongside regulated deficit irrigation (RDI), employing 50% of vine ET_c from fruit set to veraison and 100% from veraison to harvest, both independently and in combination with a biostimulant during the 2023 growing

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season. Measurements were taken for stem water potential, gas exchange, berry ripening, and yield at harvest of Cabernet Sauvignon vines. The biostimulant did not affect vine physiology, as stem water potential, gas exchange, and water use efficiency remained unchanged between treated and untreated vines. There were differences related to irrigation amount, with greater water supply leading to lower stress conditions and increased photosynthetic activity. However, this improvement came at the cost of reduced water use efficiency when compared with the 50% irrigation and RDI treatments, which had the greatest efficiency. The biostimulant increased berry and cluster weight without affecting berry ripening in combination with 50, 75, and 100% irrigation, but not with RDI. The water regime had a significant effect on juice composition, with the 50% treatment hastening berry ripening and the 100% treatment delaying it. Interestingly, RDI had the most pronounced effect on ripening, slowing down sugar accumulation and preserving organic acids.

Even though the effects of the biostimulant were limited, this study underscores that restricting water during periods when vine demand is limited represents a sustainable approach to enhance water use efficiency and delay berry ripening.

Funding Support: N/A

A Comparative Study of Traditional Drip Irrigation and Deep Underground Irrigation to Improve Water Use Efficiency

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Water efficiency is crucial for grapevine cultivation, especially in regions like California where water scarcity poses significant challenges. In 2023, a comprehensive project was initiated in Madera, CA, aimed at comparing the efficacy of traditional drip irrigation with a novel, deep-underground irrigation system in sustaining grapevine water status. The experiment comprised eight blocks, each with four vines, with four blocks irrigated conventionally using drip irrigation and the remaining four with a deep underground irrigation setup.

From June to August 2023, three water-related metrics were measured five times: stem water potential (Ψ_{stem}), stomatal conductance (g_s), and net assimilation rate (AN). Ψ_{stem} measurements showed a discernible disparity in mid-July, coinciding with peak water stress conditions. During this critical period, vines under traditional drip irrigation exhibited lower average Ψ_{stem} (-1.24 MPa) than those under deep irrigation (-1.13 MPa). Throughout the observational period, vines subjected to deep irrigation consistently demonstrated superior physiological performance. Specifically, AN remained consistently higher under deep irrigation, with an average difference of 2.6 µmol CO^2/m^2sec compared to drip irrigation. Similarly, g_s values were consistently elevated under deep irrigation, with an average difference of 65.9 mmol H_2O/m^2sec except during the initial stages of the trial.

These findings underscore the efficacy of deep irrigation in mitigating water stress and enhancing grapevine physiological functions compared to traditional drip irrigation methods. The study provides valuable insights into the potential of deep underground irrigation systems to optimize water usage and sustain grapevine health and productivity in water-scarce environments. Ultimately, such innovative irrigation approaches hold promise for improving water efficiency and promoting sustainable viticulture practices in CA and beyond.

Funding Support: N/A

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Effect of Irrigation Initiation Time on Methoxypyrazine and C13-norisoprenoid Composition in Pinot noir Wine

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This study investigated the influence of initiated irrigation time on the methoxypyrazine and C13-norisoprenoid compositions of Pinot noir wine. The experiment was conducted in three subregions of the Rogue Valley in southern Oregon for two years, and the Pinot noir vines were irrigated at different irrigation initiation times based on normalized vine water status thresholds (ΔSWP). The ΔSWP was defined as the departure of measured midday stem water potential from the calculated non-water-stressed baseline, varied from 0.2 MPa (control) to the most delayed irrigation (1.0 MPa) in five irrigation initiation times, while the irrigation amount was set at 70% ET_c, based on industry standard. Wines produced in each of the two vintages were analyzed. Methoxypyrazines and C13-norisoprenoids in the wine were analyzed by headspace solid-phase microextraction gas chromatography-mass spectrometry and stable-isotope labeled compounds were used as internal standards for quantification. The results showed that the delayed irrigation initiation time decreased the methoxypyrazine levels and increased C13-norisoprenoid levels in the wines, although the trends depended on vintage and subregion. These changes in volatile composition in response to irrigation initiation timing could affect aroma perception and overall wine quality. Determination of the optimal irrigation initiation timing can provide a guideline for grapegrowers to produce high-quality winegrapes and reserve water resources.

Funding Support: Northwest Center for Small Fruits Research Commission, USDA

Toward Real-Time Selective Harvesting and Grape Composition Mapping by Integrating Hyperspectral Sensing into Harvesters

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Understanding grape composition at harvest is vital for winemakers and growers to make informed decisions regarding grape processing and wine quality. The composition of grapes, including sugars (total soluble solids), pH, total acidity (titratable acidity), and anthocyanins, directly influences the flavor, aroma, and quality of the resulting wine. Unfortunately, grape composition varies in space because of environmental and managment factors. Implementing variable rate management can help underperforming areas recover or can help separate areas for selective production of different wines. Unfortunately, current sensing methods map grape composition indirectly. Selective harvesting cannot be implemented in real-time, requiring two separate passes to harvest the same vineyard block, increasing the time and logistic complexity of the harvest.

In this project, a novel approach using hyperspectral imaging technology mounted on the conveyor belt of a mechanical harvester was employed to assess grape composition on the go. The hyperspectral camera captured images within the shortwave infrared (SWIR) domain, ranging from 900 to 1700 nm. This spectral range allows for detecting specific molecular vibrations associated with various chemical compounds present in grapes, such as sugars, acids, and water content.

The images obtained from the hyperspectral camera were processed using advanced

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segmentation techniques to extract only the grape signal, effectively isolating the relevant spectral information for analysis. By focusing solely on the grape signal, the project aimed to minimize interference from background noise and non-grape elements, ensuring the accuracy of the composition assessment. The extracted grape signal was used to predict grape composition through previously trained machine-learning algorithms and predictions were compared to ground data collected on grape samples from 100 vines. Through this predictive modeling approach, the project seeks to develop a reliable and non-destructive method to assess grape composition in real-time during harvest. This could empower mapping and separate grape composition for on-the-fly, one-pass selective harvesting.

Funding Support: American Vineyard Foundation; California State University - Agriculture Research Institute

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Advancing Cold Hardiness Evaluation in Grapevine Mapping Populations

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Cold hardiness is an economically important trait that can significantly affect grape production and quality. Until now, there have been no reports identifying effective quantitative trait loci (QTL) for cold hardiness in grapevines. The bottleneck for QTL mapping of cold hardiness in grapes and other fruit trees is field evaluation, because plants vulnerable to cold often die in their first year of planting. To address this, our study focuses on developing a method that involves acclimating the entire mapping population in the greenhouse with controlled photoperiod and temperature, allowing comprehensive assessment of both cold-susceptible and cold-hardy genotypes using differential thermal analysis. Our primary objective is to establish a method that ensures results obtained from cold evaluation of accessions acclimated in the greenhouse have the same ranking as cold evaluation results from the same accessions under field conditions. For this purpose, a mapping population resulting from a cross between *Vitis riparia* and *Vitis vinifera* underwent cold hardiness evaluations under three distinct environments: 1) acclimated in a cold region of North Dakota that experiences temperatures as low as -35°C during winter; 2) acclimated in a greenhouse, where photoperiod and temperature were controlled; and 3) acclimated in the temperate climate of California, devoid of any cold stress. Hardiness monitoring was conducted using the widely accepted analysis method of differential thermal analysis. This research can speed up the breeding processes, as evaluation in the greenhouse can be done at any time of year and is not limited to winter. Data analysis for the study is ongoing, so the findings have not been disclosed at this stage. We anticipate completing the requisite analyses and presenting the results by the time of the Conference.

Funding Support: North Dakota Department of Agriculture Specialty Crop Block Grant

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Accelerating Molecular Breeding via Development of a Novel Multiplexed Gene-Editing Method for *Vitis vinifera*

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Genome editing can aid breeding programs dedicated to delivering genetically improved material from crops that will not be able to adapt to rapidly evolving climate change. Using the microvine system, we developed a research program to produce

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high-throughput gene-edited collections of grapevine mutants for easy gene-to-trait interrogations. To achieve this goal, we pursued the following three primary objectives: i) improve plant regeneration from *Agrobacterium tumefaciens*-mediated transformation events in somatic embryogenic grapevine cells, ii) increase the editing rate using a specific variant of the CRISPR/Cas9 protein, and iii) evaluate the potential of a new nanomaterial, carbon nanodots (CDs), to deliver multiple single guide RNAs for multiplexed editing. The approach to achieve objective 1 involves using the conditional expression of the morphogenic and synthetic gene, miRGRF_GIF4, from *Vitis vinifera*. For objective 2, we will compare the editing rate of different versions of the CRISPR/Cas9 editing system. In objective 3, we propose to identify the ratio of CD:sgRNA for optimized delivery to embryogenic grapevine cells. Zinc-functionalized CDs have been selected for their ease of synthesis and zinc's affinity for nucleic acids. If we achieve our goals, we will give the scientific community a new tool for generating KO mutant collections that could expand beyond the grapevine model.

Funding Support: USDA NIFA

Spray-Induced Gene Silencing (SIGS) with Carbon Dots for Enhanced RNA Delivery in Grapevine

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Spray-induced gene silencing (SIGS) is a recent technology that enhances a plant's immune system. It involves the ectopic application of double-stranded RNAs (dsRNAs) to trigger a plant's defense mechanism, known as RNA-interference (RNAi). Unlike traditional methods relying on the generation of transgenic plants, SIGS offers a rapid and targeted disease management solution. By transiently enhancing RNAi, it eliminates the need for resources and time-consuming development of resistant plants. However, SIGS application in tree crops encounters significant challenges, including limited cellular uptake and environmental stability of dsRNAs. To address these limitations, we explored an innovative approach that combines SIGS with branched polyethyleneimine-functionalized carbon dots (CDs) as nanocarriers, which enhanced dsRNA stability and cellular uptake. We developed a cost-effective, microwave-assisted protocol to synthesize 10 nm monodispersed CDs, that achieved >50% protection of dsRNA against RNase III degradation at a 200:1 (w/w) ratio. These CDs effectively improved cellular uptake, demonstrated by the successful delivery of CD-complexed fluorolabeled-dsRNAs (Cy3-RNA) into intact plant tissue-cultured cells. To prove the applicability of this formulation *in planta*, we sprayed Cy3-RNA with CDs on the abaxial surface of greenhouse grapevine leaves via low-pressure spray. Fluorescence microscopy revealed penetration of Cy3-RNA into stomata and neighboring cells. Additionally, the efficacy of CD-complexed dsRNA in triggering RNAi was validated using a 21-mer dsRNA targeting eGFP in eGFP-expressing microvines. Notably, no cytotoxic effects were observed following CD application. Ongoing research quantitatively compares eGFP expression of microvine leaves sprayed with CD-complexed and naked dsRNA through qPCR, to demonstrate the improvement in nucleic acid delivery associated with CDs. This innovative approach aims to overcome critical barriers for dsRNA delivery, particularly those associated with cell wall-related constraints in plants. Through optimization of RNAi-based defense strategies in grapevine, this research contributes to advancement of sustainable viticulture practices.

Funding Support: Erath Family Foundation, Oregon Wine Board

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Simulating Natural Wildfire Smoke Events in a Vineyard: Challenges and Solutions

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Changing climate is increasing wildfire events across the United States, exposing some of the most productive vineyard regions of the world to wildfire smoke. Wildfire smoke contains thousands of chemical compounds that can diminish growth and productivity of grapevines, delay ripening, and impart an offensive flavor in wine referred to as "smoke taint". However, these effects are contingent upon numerous factors, including time and distance of the smoke event, smoke concentration, fuel type, and growth stage of the vine. Field experiments involving custom-built smoking chambers, described in previous research, have provided inadequate information on how to simulate a natural wildfire event and achieve a predetermined smoke concentration within these chambers. Therefore, a field trial was conducted at the Southern Oregon Research and Extension Center in the Rogue Valley AVA, where chambers constructed from PVC frames and covered with transparent plastic sheets were used to cage six vines within a vineyard row. Smoke was generated by burning Douglas fir pellets in a battery-operated grill and channeled into the chambers, with smoke treatments including an open-air control, a chamber control, and two levels of smoke treatments (low and high PM 2.5 at 1200 and 4000 $\mu g/m^3$, respectively), to mimic typical concentrations recorded during low and moderate smoke years, converted over a two-hour period. A battery-operated mixing fan was installed inside each smoking chamber to distribute smoke evenly and regulate vine stomatal action. Smoke levels inside the chambers were monitored in real time using an air quality monitor and were regulated using valves attached to inlet tubes. Stable predetermined smoke concentrations were achieved inside chambers within 20 min of experiment initiation. Temperature and relative humidity inside the chambers were monitored in real time with sensors. This research highlights key technical challenges for simulating wildfire smoke events at a vineyard scale and demonstrates methods to achieve this goal.

Funding Support: USDA-NIFA-SCRI

Development of a High-Throughput Phenotyping Method to Assess Boron Tolerance for Breeding Programs

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Boron serves as an essential micronutrient for plants, yet at elevated concentrations, it becomes toxic. In grapevines, boron toxicity triggers leaf senescence, reduces yield, and can ultimately lead to plant death. Among different grapevine species, there is variation in the ability to exclude boron from the shoots. This trait is a crucial component of boron tolerance and a valuable target for rootstock breeding. Our research leverages an extensive grapevine germplasm collection at the University of California, Davis, which includes wild *Vitis* species and breeding selections with natural variation in boron tolerance. Considering the low throughput and high costs associated with current methods for assessing boron tolerance, we are developing a new high-throughput phenotyping pipeline that integrates hyperspectral proximal

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sensing and machine learning modeling to predict boron content in different plant tissues. This method will significantly reduce the time required to identify grapevines with improved boron tolerance and eliminate the need for plant tissue collection. We will use this method to screen a large diversity panel genotyped with approximately one million molecular markers for genome-wide association mapping of boron tolerance traits and to identify genes responsible for boron exclusion. This research will provide insights into the adaptive mechanisms of grapevine boron tolerance and facilitate development of more resilient rootstocks for viticulture through the application of phenomics and molecular breeding.

Funding Support: Vaadia-BARD Postdoctoral Fellowship and University of California, Davis

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Testing the Application of a Novel Technology for Assessing Grape Maturity using Spectrometry

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The Niagara region wine industry in Ontario, Canada, is already experiencing the effects of climate change, and more extreme weather events are expected. It is predicted that the changing climate will result in significant consequences for quality wine production. Consistent monitoring of grapes will help maximize their enological potential. However, most monitoring techniques are either destructive to the grapes or time-consuming (e.g., UAV flights). Past research on soft fruits and Chardonnay grapes suggests that the use of the visible light spectra can predict fruit/berry ripeness in a time-efficient and non-destructive manner.

PRecent advancements in compact and inexpensive technology for monitoring individual woody plant health and growth conditions, called TreeTalker, have been adapted to vines (TreeTalker Wine; TTW). This adapted technology allows continuous, real-time monitoring of grape status using spectrometry. Here, we present results from experiments that tested TTW's ability to monitor grape veraison. We developed empirical relationships between spectral signatures measured by TTWs and berry total soluble solids (TSS) and investigated the possibility of using TTWs to predict berry TSS *in situ* and continuously on the vine until harvest.

Funding Support: Sources are University of Waterloo, Nature 4.0, and Ontario Grape and Wine Research Inc.

Initiation of a Three-Year Survey to Characterize the Distribution and Abundance of Nematodes in Oregon Vineyards

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Winegrapes are an excellent host for numerous plant-parasitic nematodes and previous work has characterized nematode presence in Oregon vineyards. However, little work has been done to catalog the diversity of nematode species found in OR vineyards since the mid-1990s. The OR winegrape industry has expanded significantly since then, growing to nearly 45,000 acres. The cultivation of winegrapes now occurs on land with diverse usage histories ranging from forest and native pasture to former fruit and nut orchards. Therefore, this three-year survey was designed

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to identify the distribution and abundance of plant-parasitic nematodes across OR vineyards. The survey was started in the fall of 2023 and will continue through the spring of 2025 with the intention of capturing nematode diversity throughout the diverse viticultural geography of the state across three years. Samples will be taken from the predominant winegrowing areas of the state, including the North and South Willamette, Colombia, Rogue, and Umpqua regions. Fall 2023 samples were collected from 43 vineyard blocks across the state, distributed relative to planted acreage. Plant-parasitic nematodes were identified in 79% of surveyed vineyards blocks and in all five sampled regions of the state. *Xiphinema* spp. were identified in 51% of sampled blocks and in all five regions. Samples from the Colombia region yielded high abundance of *Meloidogyne* spp., due to the sandy soils. Samples from the Rogue region exhibited a high abundance of *Mesocriconema* spp., as they were present in 75% of vineyard blocks surveyed. This project aims to update and improve upon the previous statewide nematode survey from the mid-90s to provide better baseline information to the OR wine industry. The baseline data will benefit the industry by providing guidance for future studies on vineyard health and productivity, rootstock selection, and management of nematodes.

Funding Support: USDA-ARS Northwest Center for Small Fruits Research

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Mitigation of Grapevine Red Blotch Disease Effects using Potassium and Inducers of Plant Resistance

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Grapevine red blotch disease (GRBD), caused by the grapevine red blotch virus (GRBV), manifests foliar symptoms consisting of interveinal blotching on the leaves and is associated with reduced photosynthesis and carbon translocation, leading to lower sugar and anthocyanin levels in the fruit. Previous research established the mechanistic role that potassium (K) plays in facilitating sugar transport within the phloem. It has also been reported that GRBV-infected grapevines that are asymptomatic do not exhibit the same fruit quality reductions as symptomatic grapevines, suggesting that increased plant defense response could mitigate some of the effects caused by GRBD. Therefore, it was hypothesized that foliar- and/or soil-based applications of K and products that elicit systemic acquired resistance (SAR) or induced systemic resistance (ISR) may mitigate the fruit quality effects of GRBD. In 2023, two rates of soil-applied K and a single rate of foliar-applied K were evaluated using a split-plot design on GRBV-infected Cabernet franc and Merlot. In both cultivars, there was a significant effect of foliar K on total soluble solids (TSS; $p = 0.046$) and a significant effect of soil K on juice pH ($p = 0.001$), where increased foliar and soil K rates resulted in higher juice TSS and pH, respectively. In addition, a significant increase in sugar per berry was observed in response to increased soil-applied K in Merlot only. Two SAR/ISR products, Regalia and Actigard, were applied in a separate randomized complete block design on separate Cabernet franc vines. These products had a significant effect on yield ($p = 0.032$), with a 70% increase in Regalia-treated grapevines and an 18% decrease in Actigard-treated vines. Although some effects of K and plant resistance inducers were evidenced in this study, repeated evaluation over multiple years is necessary to understand the long-term effects of these treatments.

Funding Support: Oregon State University, Agriculture Research Foundation (OSU ARF)

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Optimizing Biofungicide Use for Control of Grapevine Diseases on the Central Coast of California

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Biofungicide efficacy in grapevine disease management is enhanced by selecting the right active ingredients and modes of action, optimizing application timing and understanding the pathogen's biology, then integrating them properly into fungicide programs. The efficacy of biopesticides in vineyard production is influenced by local climate and environmental conditions. The objective of this study was to investigate the efficacy of biofungicides applied at different intervals in controlling powdery mildew and Botrytis bunch rot, and their effect on grape quality. The study was conducted in the San Luis Obispo Coast AVA on mature Chardonnay vines. Twelve fungicide programs were designed, with three biofungicides containing different active ingredients and modes of action, application intervals, and integration strategies. A grower-standard program and an untreated control were also included in the experiment. Fungicide applications started at bloom in May 2023 and finished at veraison in August 2023. Fungicide program efficacy was determined based on disease incidence and severity. Yield was measured by weighing all clusters from each plot. Berry chemistry (total soluble solids, pH, and titratable acidity) was assessed following standard laboratory protocols. Biofungicide active ingredient and mode of action significantly affected program efficacy, while the interval of application did not. All programs integrating biofungicides with synthetics had efficacy comparable to the grower standard which, with the exception of one biofungicide stand-alone program, resulted in significantly higher powdery mildew and Botrytis bunch rot disease control than biofungicide stand-alone programs. Yields were also significantly greater in these programs. This suggested that when designing a program with biofungicides in this AVA, integration with synthetics can significantly improve the efficacy of the programs. Berry chemistry was affected significantly by the fungicide program, but results did not necessarily correlate with their efficacy.

Funding Support: CDFA-Specialty Crop Black Grant Program and CSU ARI

Summer Bunch Rot and Sour Rot Management on Table Grapes in the San Joaquin Valley

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Sour rot (often known as summer bunch rot) is a disease complex that can be caused by multiple fungal species, including several *Aspergillus* spp., *Botrytis cinerea*, *Cladosporium* spp., *Penicillium* spp., and *Rhizopus* spp. or by yeasts, bacteria, and Drosophila flies. It is commonly found in the central and southern San Joaquin Valley of California due to the typically warm climate. Because this complex involves multiple causal organisms, it isn't easy to control and multiple strategies are likely needed. To help determine the efficacy of disease control options, a field trial was conducted in two commercial table grape vineyards, in Delano on Red Globe and in Bakersfield on Autumn King, comparing seven spray treatments to an untreated control. Each treatment was replicated five times per site and sprays were repeated four times (bloom, pre-close, veraison, and preharvest) during the 2023 growing

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season. Shortly before harvest, each plot was evaluated for disease incidence and severity. A representative number of harvested bunches were stored in commercial cold storage (0°C, 90 to 95% RH) for five weeks, then rated for disease incidence and severity to determine the treatment efficacy during cold storage. Differences in natural disease pressure were observed between the two vineyards, with greater pressure in the Red Globe vineyard than in the Autumn King vineyard. The Elevate/Luna Experience/Switch/Vacciplant treatment was shown to be the most effective at reducing disease incidence in Autumn King, but there were no significant differences seen in Red Globe. After five weeks in cold storage, however, no product was significantly different from the control in incidence or severity in Autumn King, while the Pristine/Luna Experience/Elevate/Scala treatment resulted in significantly less incidence of infected bunches than the untreated control in Red Globe. Based on these findings, we plan to renew the project for another year to continue product evaluations.

Funding Support: California Table Grape Commission

Understanding the Mechanistic Basis of the Red Blotch Virus Infection for Better Disease Management

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Grapevine red blotch disease (GRBD), caused by Grapevine red blotch-associated virus (GRBaV), is a severe concern to grapegrowers and winemakers in major grapegrowing regions worldwide. One key aspect of all viruses, including GRBD, is their intimate association with cell components and anomalous structures following infection. This study aimed to analyze symptomatology, vine function, fruit quality, and ultrastructure of various tissues, and to document the relationship of ultrastructural cytopathology with GRBaV infection in Pinot noir, employing multiple microscopy techniques. The infected vines exhibited typical red blotches in leaves, with pinkish-red-colored veins, without rolling off the margins at the onset of ripening. The infected vines developed clusters of hens and chickens and altered seed morphology. Conversely, the healthy seeds were pyriform with a distinct beak. The infection significantly changed the composition of primary and secondary metabolites desired for making wine. Since postveraison berry development and ripening rely on phloem influx, the altered metabolism indicated disruption of the phloem pathway, either in the source leaf or in the berries. Furthermore, the infection modified the browning process, altering periderm formation in infected canes. Nonetheless, the infected green stem turned brown during the cold acclimation process. While the infected vines maintained primary leaf anatomical organization, their chloroplasts underwent significant ultrastructural changes, ranging from complete dismantling to massive accumulation of starch and plastoglobuli development in the chloroplasts and tannins in the cytoplasm. The results demonstrated that structural integrity is vital to maintaining normal metabolism of the grapevine, providing new insights into implementing innovative approaches for GRBD management.

Funding Support: Northwest Center for Small Fruits Research (NCSFR)

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Advancing Vineyard Irrigation in the San Joaquin Valley, California, with Hyperspectral-Based Plant Water Status Mapping

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Remote sensing has emerged as a valuable tool to enhance the precision of water supply budgeting using both spectral and spatial data. A study conducted in a *Vitis vinifera* L. cv. Cabernet Sauvignon vineyard in the San Joaquin Valley, California used a variable rate automated irrigation system across 12 distinct water regimes replicated in four randomized sets, resulting in 48 experimental zones. The primary objective of this experimental setup was to introduce variability in grapevine water status, facilitating the generation of a comprehensive data set for modeling purposes. Over the course of the growing season, spectral data within these zones were collected using a near infrared (NIR) - short wavelength infrared (SWIR) hyperspectral camera (ranging from 900 to 1700 nm) mounted on an unmanned aircraft vehicle. Given the significant water absorption characteristics within this spectral range, the sensor was deployed to evaluate grapevine stem water potential, Ψ_{stem}, a standard metric for assessing plant water status, from pure grapevine pixels in the hyperspectral images. Concurrently, Ψ_{stem} values were measured in the field from bunch closure to harvest and subsequently modeled using machine-learning techniques, leveraging the remotely sensed NIR-SWIR data as predictors in both regression and classification frameworks (with classes representing varying levels of physiological water stress). Hyperspectral images underwent conversion to bottom-of-atmosphere reflectance using standard ground panels and the quick atmospheric correction method, with ensuing results subjected to comparative analysis. The most effective models used ground panel-derived data and predicted Ψ_{stem} values with an R^2 of 0.54 and an RMSE of 0.11 MPa, as estimated through cross-validation. Additionally, the optimal classification approach achieved an accuracy rate of 74%. This endeavor aims to pioneer novel methods for precise monitoring and management of irrigation in vineyards, while concurrently furnishing valuable insights into the physiological responses of plants to deficit irrigation practices.

Funding Support: American Vineyard Foundation, CSU-Agriculture Research Institute

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Establishing Baseline Data for Carbon Isotope Discrimination as an Effective Indicator of Rootstock Drought Tolerance

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Drought is an existential issue for growers across most American grape acreage and in grapegrowing regions throughout the world. Adaptation to drought therefore has always been a crucial consideration for growers. One strategy to manage grapevines under water-limiting conditions is to use drought-tolerant rootstocks. However, the grapevine rootstocks that are in use today were bred primarily for *Phylloxera* tolerance, so their drought responses have not been sufficiently examined. Plants, under drought stress, tend to discriminate against the lighter carbon isotope (C^{12}) for the heavier one (C^{13}) during photosynthetic carbon assimilation. This physiological trait – carbon isotope discrimination ($\delta^{13}C$) – has been used as a proxy for water status in grapevines. Therefore, this three-year-long research project was initiated to evaluate drought tolerance of 10 commonly-used rootstocks using $\delta^{3}C$, vine phenology, physiology (stomatal conductance [gsw] and stem water potential

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[Ψ_{stem}]), yield, and fruit quality (juice total soluble solids, pH, and titratable acidity [TA]) in a young, replicated field trial at the Southern Oregon Research and Extension Center in the Rogue Valley AVA. In the pilot year of the study (2023), all rootstocks were irrigated uniformly and baseline data were recorded. A significant rootstock effect was observed on phenology (budbreak, berry set, and veraison), gsw measured at veraison, vine yield, cluster weight, berries per cluster, and TA in the juice. In subsequent years of this trial, irrigation will be withheld from half of each experimental plot (scion-rootstock-block) postbloom. Under drought stress conditions, differences between rootstocks may be magnified and can be capitalized to highlight specific strengths and weaknesses of each genotype. Therefore, the main aim of this project, identification of drought-tolerant rootstocks and establishing selection and management criteria, will be satisfied at the end of this three-year-long field trial.

Funding Support: Oregon Wine Board (OWB), California Grape Rootstock Improvement Commission (CGRIC)

Warmer but Longer Cold Air Exposure Can Hurt

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Extreme cold events occurred in the Okanagan Valley, British Columbia, in December 2022 and January 2024. At Summerland Research and Development Centre, the lowest temperature reached -22.7°C in 2022 and -25.2°C in 2024. In 2022, grapevines were exposed to air temperature below -18°C for 42 hrs. In this area, the lethal temperature of buds was monitored regularly by differential thermal analysis. Before the 2022 cold snap, the lethal temperature to kill half of Merlot buds was -22.6°C in a commercial vineyard. The lowest temperature recorded was -21.5°C in the same area. With the known lethal and lowest temperatures recorded, the estimated bud damage rate was <50% at this site. However, the observed damage rate was 77%. This suggested that exposure time might be critical. The procedure of differential thermal analysis was modified to simulate 24 hrs exposure at -14, -16, -18, and -20°C. The canes were sampled from a Merlot vineyard in Summerland, BC in 2024 before the cold event and stored at 1 3 3°C in a walk-in cooler until use. Forty buds were tested in each treatment. When the low temperature exotherm was detected, the timestamp was recorded simultaneously. After the 24 hrs treatment at constant temperature, the freezer temperature was ramped down to -33°C at a rate of 4°C/hr to kill all surviving buds. The cold hardiness of an additional 54 buds was also evaluated by the standard method. The results showed the lethal temperature was at -22.4°C measured by the standard method. However, six hours of exposure at -18°C was sufficient to damage 50% of the buds. The preliminary data suggests shorter exposure time is required at lower temperature to inflict bud damage. The exposure time should be considered when estimating bud damage rate.

Funding Support: Agriculture & Agri-Food Canada

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Characterizing Root System Architecture Traits for Drought Tolerance in Grapevine Rootstocks

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To reduce the effects of climate change on viticulture, breeding more drought-tolerant rootstocks is a promising, but difficult, strategy due to limited information on traits and genes of interest. Our goal is to use an F1 population produced from a drought-tolerant and a drought-sensitive rootstock (110 Richter 101-14 MGT) to identify molecular markers for root system architecture (RSA) traits in grapevine. Neutron radiography is an imaging technique that can non-destructively visualize entire small root systems. We subjected plants to two-week dry-down and rewatering treatments and used neutron radiography and new semi-automated root classification software to measure RSA. Here, we present preliminary results for a subset of 45 genotypes. We will complete measurements across the entire population and use genotyping by sequencing (GBS) to link RSA traits to molecular markers by next summer.

The 45 genotypes varied significantly ($p < 0.001$) in nearly all RSA traits, including total root length, mean root angle, aspect (total horizontal/vertical extent), root growth rate, and change in depth during drought. Roots were significantly steeper rooted and thicker in 110 Richter than 101-14 MGT, suggesting these are especially important traits for drought tolerance. Across genotypes, a more vertical aspect was correlated with a steeper root angle ($r^2 = 0.47$) and thicker mean root diameter ($r^2 = 0.09$), but independent of root system size and fibrosity (root system length/# of tips) ($p > 0.05$). Altogether, these findings suggest that this population is well-suited to identify genetic associations with RSA, and that selecting for a more vertical aspect, steeper root angles, and thicker roots could enhance drought tolerance in grape rootstocks, though more work is needed to evaluate how these phenotypes perform under field conditions. Genetic associations are pending current experimentation results.

Funding Support: California Department of Food and Agriculture

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Evaluating Nitrogen, Potassium, and Magnesium Fertilization Effects on Vine Nutrient Status and Productivity in Western Oregon

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Mineral nutrients play a crucial role in ensuring optimal vine growth and high-quality fruit production. This study investigates the effects of nitrogen (N), potassium (K), and magnesium (Mg) fertilization on vine nutrient status, productivity, and fruit composition in western Oregon vineyards over three years (2021 to 2023). Three trials were conducted: one focused on N in Chardonnay, while the other two addressed deficiencies of K and Mg in separate Pinot noir vineyards. Each trial had treatments replicated four times using a randomized block design. The N trial involved three rates of soil-applied N (no N, 20 lb N/acre, and 40 lb N/acre), while the K trial included both soil (no K, 200 lb K/acre, and 400 lb K/acre) and foliar fertilizer (3.3 lb K/acre) treatments. The Mg trial focused solely on foliar applications (no Mg, 3 lb Mg/acre, and 6 lb Mg/acre). Key findings revealed minimal effect of N on growth and fruit metrics, yet it positively influenced vine N status, with

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effects varying across different tissue types and over time. Additionally, juice yeast assimilable nitrogen levels increased as the N application rate increased. Notably, soil-based K application had more visible effects than foliar application or no K on vine growth; vine K status across leaf blade, petiole, and dormant stem; and juice pH by the end of the third year. The Mg trial had limited effects on vine growth and productivity, although leaf blade Mg increased with greater Mg application rates. Petioles did not respond significantly across the years. However, foliar Mg application reduced the severity of symptomatic leaves, particularly at high and medium application rates. These findings contribute to our understanding of nutrient management in western OR vineyards and are being used to establish critical nutrient levels tailored to specific grape varieties in the region and nationally as part of the High Resolution Vineyard Nutrition Project.

Funding Support: National Institute of Food and Agriculture-Specialty Crop Research Initiative Coordinated Agricultural Projects (CAP) grant project award number: 2020-51181-32159

Enhancing Vine Resilience and Delaying Ripening: Novel Late-Season Practices for Grape Production in Central California

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Global warming is significantly compromising grape production in regions like central California. Late-season practices aimed at reducing the efficiency of the photosynthetic apparatus might be the solution to improve vine resilience and delay ripening. In this trial, an untreated control (C) was compared with applications of pinolene (P) and diatomaceous earth (D). A fourth treatment involved shoot twisting (T), seeking to achieve the benefits of traditional topping by breaking xylem tissue without overexposing clusters. Leaf area, light interception, midday stem water potential, and gas exchange were measured over the 2023 season. Berry ripening was followed and grape production at harvest was evaluated. Twisting determined the desiccation of the upper portion of the shoot, reducing leaf surface while maintaining a low level of irradiance in the fruiting zone. Stem water potential results showed that D, and especially T, partly alleviated stress conditions, while P led to a worsened vine water status. D had the most pronounced effect on gas exchange, especially transpiration and stomatal conductance, thereby leading to higher water use efficiency trend in the treated vines. D and T fruit exhibited greater berry weights during the first half of the season, likely due to the improved water status, while P berries had reduced weight. All applied treatments significantly affected berry ripening, resulting in increased titratable acidity and lower must pH, without compromising crop yield at harvest.

This trial highlights that innovating traditional, easily mechanizable practices such as topping, and introducing new products like diatomaceous earth can be effective techniques to mitigate summer heat stress and delay berry ripening, particularly in hot, dry regions such as CA's Central Valley.

Funding Support: N/A

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Applications of Foliar Nutrients and Plant Growth Regulators can Improve Berry Firmness and Color in Red Table Grape

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Tablegrape growers in the San Joaquin Valley, California face a significant challenge: berries of some red varieties lose firmness before harvest. This issue, known as preharvest fruit softening, has inflicted significant economic losses upon the CA table grape industry and appears to stem from disconnection between berry firmness decline and color development during ripening. Potential remedies include enhancing berry color development and extending the period during which berries maintain firmness after reaching maturity (18 to 19 Brix). In 2023, we evaluated whether cluster thinning, foliar nutrient applications (Ca, Mg, and orthosilicic acid), or foliar sprays of plant growth regulators (24-epibrassinolide, ethylene, and abscisic acid) could enhance berry firmness and color during ripening and ultimately increase marketable fruit at harvest. The trial was conducted in McFarland, CA in a Krissy vineyard that previously experienced preharvest fruit softening. We found that foliar sprays of Ca and Mg every two weeks from bloom to veraison resulted in firmer berries, but slower color development. Biweekly applications of orthosilicic acid during the same period improved berry color but had only minor effects on berry firmness. Applying 24-epibrassinolide at bloom, fruit set, and veraison increased berry firmness during ripening without affecting berry color. Application of ethylene with abscisic acid at veraison enhanced berry color, but compromised berry firmness. Cluster thinning at 10% at fruit set did not affect berry color or firmness. Neither foliar sprays nor cluster thinning altered harvestable yield and total yield at the first harvest. Due to an unexpected storm in late August and subsequent severe bunch rot, we were unable to collect more data. Even so, this study showed the potential of applying Ca and Mg, orthosilicic acid, and 24-epibrassinolide to mitigate preharvest fruit softening in red table grapes.

Funding Support: UC ANR and industry donation

Identification of 2-methylisoborneol as an India Ink-Like Off-Flavor in Syrah (*Vitis vinifera* cv.) Wine

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The objective of this study is to reveal the cause of an India ink-like off-flavor in Syrah berries and wine made from grapes grown in a vineyard in Japan in 2020 and 2021. Microbial contamination was suspected as the cause because this off-flavor was detected not only in wine, but also in Syrah berries. Thus, the study was conducted to identify the fungus and the specific flavor compound(s) responsible.

Grape berries collected during harvest, with a similar India ink-like odor, were used for this study. Fungi were isolated from these berries by rinsing the surface and culturing the rinse water on an agar medium. About 20 fungal strains were isolated and one released a strong India ink-like odor. This strain was identified as a *Coniella* spp., known as grape white rot fungus. Furthermore, we found that the Syrah berries infected with this strain released the India ink-like odor, while control berries did not. The aroma compounds of infected/non-infected berries were analyzed using the SIM/Scan mode of gas chromatography-mass spectrometry.

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These berries were incubated in a closed container and the odorous compounds were collected in a TENAX GC collection tube (P/N: 223-57102-91) by nitrogen purge. Several compounds were identified, such as fenchol, 2-methylisoborneol (MIB), and benzofuran, that are specific to the infected berries. It was considered that MIB is the candidate compound for the cause of this off-flavor because of its camphor-like odor.

The study concluded that the India ink-like off-flavor is caused by generation of MIB during infection by *Coniella* spp. This is the first report that *Coniella* spp. can produce MIB. These new insights are expected to contribute to production of high-quality grapes and wines.

Funding Support: Kirin Holdings Company, Limited

Autonomous Identification of Grapevine Varieties in Sentinel 2 Satellite Imagery

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The development of new varieties has played a significant role in maintaining a strong and stable food supply. New plant varieties enable consumers to enjoy diverse, safe, nutritious, and abundant food and are protected under the Plant Variety Protection Act. The protection of intellectual property encourages continued investment in research and development and encourages bringing innovations to farmers to help them cope with changing conditions, from the climate to the markets. Protected plants can still be vegetatively propagated illegally. This undermines investment in research and development of new varieties and sustainable agricultural technologies.

This study aimed to investigate the ability of machine learning to identify grapevine varieties in Sentinel 2 satellite imagery. Our preliminary results, employing dimension reduction techniques alongside rigorous cross-validation, show an accuracy rate of >84% on >400 accessions within a single location. This accuracy underscores the efficacy of our approach in effectively discriminating among a diverse array of grapevine varieties present within the ranch.

Funding Support: N/A

Use of Reverse Osmosis, Immobilized β-glucosidase, and Adsorption as Remedy for Smoke-Affected Wine

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Every year, wine producing countries are affected by smoke from wildfires. Wine made from grapes that have been exposed to smoke from wildfires can have unpleasant burnt, leather, smoky, and other sensory attributes. Smoke-related volatile phenols that play a role in the smoky characteristics exist as free phenols and sugar-bound glycosides. Free volatile phenols contribute to smoky aromas in wine. After wine fermentation, acid-mediated hydrolysis can release bound phenols, leading to increased smoky aromas. To improve the quality of smoke-affected wines, both free and bound smoke-related phenols must be removed or reduced. There are no effective means to reduce both free and bound smoke phenols in affected wines.

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Use of reverse osmosis (RO) with solid phase adsorption reduces free-smoke related phenols but not bound-smoke related phenols. This can result in smoke recurrence after some time. This study evaluates the use of immobilized β-glucosidase to release bound-smoke related phenols in wine and permeate which can then be removed by RO and suitable adsorption technique. Smoke-affected wines were treated using RO to create a permeate containing the phenol glycosides. β-glucosidase was immobilized in cross-linked chitosan-silica microspheres. Both original wine and permeate were subjected to hydrolysis by immobilized β-glucosidase at different temperatures for four hours. Released volatile phenols can then be removed by adsorption using activated carbon. Enzyme hydrolysis of phenol-glycosides was dependent on sample type (wine or permeate), temperature, and incubation period. In both wine and permeate, 35°C had highest hydrolysis of phenol-glycosides (21.47% and 33.82%, respectively) with a high hydrolysis rate within the first two hours of incubation. This combination of immobilized enzyme with RO and adsorption can improve the quality of smoke-affected wines and greatly reduce losses incurred by grapegrowers in the case of wildfires.

Funding Support: USDA Washington State Wine Agricultural Research Service

Comparison of Freeze-Killed versus Freeze-Dried Leaves for the Production of Frost Tainted Cabernet Sauvignon Wines

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A commercial freeze-dryer was used to emulate the effects of freeze-killed leaf material on Cabernet Sauvignon wines in 2023. Fresh leaves were collected from a vineyard in Sunnyside, WA one month prior to harvest, and freeze-killed leaves (FK) were collected at harvest. The fresh leaves were subjected to freeze-drying (FD). The FK and FD leaves were added directly to the must prior to fermentation at two rates (0.9 and 3.6 g/kg must) including a control (0 g/kg must). Basic wine chemistry was unaffected by the additions. Untargeted solid-phase microextraction gas chromatography-mass spectrometry was used to identify major aroma compounds present. Tentatively identified compounds were evaluated statistically. Twenty-three compounds were found to vary significantly based on the treatments. Alcohols were significantly reduced by both leaf treatments. Terpenoids and norisoprenoids significantly increased with increased leaf dosages. A previously-suggested frost taint marker, 6-methyl-5-hepten-2-ol, which smells like coriander was only found in the FK-treated wines. Esters significantly increased in both treatments, consistent with dosage. Phenolics were measured in the wines using the Adams-Harbertson assay. Counter to previous results, wines made with both FK and FD leaves had significantly more phenolics and anthocyanins were unaffected. With the exception of the single frost taint marker, the FD treatments emulated the FK-treated wines.

Funding Support: Washington Wine Commission

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Effect of Cold Storage and Partial Grape Drying on Acidity and Aroma Precursors of Cold-Climate Interspecific Hybrids

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Nordic wine regions face challenges related to seasonal climate variations, including cold nights from late August onward that may affect berry ripening, preventing the breakdown of malic acid and hampering the accumulation of aroma precursors. With climate change, large climate fluctuations during the season further contributes to variable levels of ripening in northern vineyards. In *Vitis vinifera*, partial grape drying and cold storage have been shown to decrease the acid content of berries and increase their concentration in aroma precursors, including thiol precursors. Enological techniques to improve berry quality are understudied in interspecific hybrid grapes. In this experiment, we evaluated the effect of postharvest processes such as cold storage (10°C) and partial grape drying at two temperatures (20 and 30°C) on the organic acid and thiol precursor content of two white (St. Pepin, Frontenac blanc) and two red (Frontenac, Marquette) *Vitis* sp. varieties, during 12 and 24 days. Compared to berries prior to treatment, higher temperature (20, 35°C) lowered the titratable acidity (TA) in Frontenac, but not in Marquette. In contrast, cold storage increased TA in Marquette and St. Pepin. Only the highest temperature (35°C) decreased TA in Frontenac blanc and St. Pepin.

Aroma precursors are currently being analyzed so results will be available on the poster.

Funding Support: AAFC, CGCN, MITACS

Using Cover Crop to Mitigate the Effects of Winery Wastewater Application on Soil, Grape, and Wine Quality

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The San Joaquin Valley is facing drought and saline conditions, both of which have adverse effects on grapevine growth. Approximately 3000 to 5000 L wastewater are generated per metric tonne of crushed grapes annually, posing risks to vineyard soil physical and chemical properties, and ultimately affecting grape and wine quality. This study aims to examine the effects of cover crops on soil properties, determine the effect of cover crops on grape and wine quality, and promote effective water and soil management within the framework of sustainable viticulture.

Eight-year-old Ruby Cabernet vines from a commercial vineyard in Fresno, California were used for this study. The experimental design was a randomized complete block design with eight treatments replicated four times. The treatments were: a control (no-till with residential vegetation), tilled, UC 937 barley, WB patron wheat, Pacheco triticale, Sierra oats, rye grass, and Dairyland magnum salt alfalfa. Each experimental unit consisted of one vine row, a quarter-mile in length. The vineyard was furrow-irrigated with wastewater before planting the cover crop.

Results from the two years of the study revealed significant effects of cover crops on soil chemical compositions. Barley, oat, rye grass, triticale, and wheat sequestered carbon at higher levels (200 to 400 kg/acre) than the control. Oat, rye grass, triticale, and wheat absorbed nitrogen at higher levels (6 to 10 kg/acre)

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than the control. Barley and rye grass absorbed higher sodium content than the control. Triticale and wheat reduced soil electrical conductivity. Rye grass, triticale, and wheat resulted in lower soil sodium. Yield components exhibited no difference among treatments. However, preliminary wine sensory analysis has shown that the treatments may influence both aromatic properties and color. The project is ongoing and additional data will be provided after completion of the third-season trial.

Funding Support: Agricultural Research Institute (ARI)

Evaluation of Ripe-Fruit-Like Aroma in Red Wines using an Automated Omission System

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Aroma is one of the most important criteria used to evaluate the quality of wine, and >1000 aroma compounds have been detected in wine. However, it is difficult to evaluate the combined effects of wine aromas originating from complex mixtures of volatile compounds, and to identify the key aroma compounds creating a specific nuance in the wine. An automated omission system was developed by Shimadzu Corporation to clarify the combined effects of multiple volatile compounds. As a configuration of the system, the gas sampling device and mass spectrometer are connected to divergent ends of the gas chromatography (GC) column. The volatile compounds in the injected samples are separated by GC and the targeted compound can be isolated to be analyzed by mass spectrometry, while the remaining compounds can be collected in a bag using the gas sampling device. The omission system allows us to evaluate whether the targeted compound is essential for total aroma nuance.

In this study, we verified whether this omission system can evaluate complex mixtures of volatile compounds in various types of red wine. At first, we evaluated the effect of ethyl hexanoate on the aroma of Muscat Bailey A wine, an indigenous grape cultivar in Japan. The volatile compounds in the wine were collected using the dynamic headspace method and injected into the omission system. Three sample bags were prepared: (1) one filled with all the volatile compounds of the wine, (2) one filled with all compounds but ethyl hexanoate, (3) one in which ethyl hexanoate was added back into bag 2. The results of sensory evaluation of these sample bags demonstrated that ethyl hexanoate was responsible for the "ripe-fruit-like aroma" in Muscat Bailey A wines. We will also report the impact of ethyl hexanoate on the aroma of two other wine varieties, Pinot noir and Cabernet Sauvignon.

Funding Support: Kirin Holdings Company, Limited

Surface-Enhanced Raman Spectroscopy for Quick Smoke Exposure Assessment

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Smoke exposure in grapes has become a major concern for the United States wine industry. Smoke exposure can lead to off-aromas with a smoky, ashy, and medicinal characteristic. When grapes are exposed to heavy smoke, volatile phenols and other

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volatile compounds are absorbed by the grapes and subsequently bind to the sugars to form glycosides. When the grapes are fermented, the off-aroma compounds are released from their bonded glycosides, contaminating the produced wine. The volatile phenols, including guaiacol; 4-methylguaiacol; and *m*-, *o*-, and *p*-cresol, are the most studied compounds related to smoke exposure. These compounds are typically analyzed by gas chromatography-mass spectrometry (GC-MS), providing information on smoke exposure and potential smoke taints. Due to the costly and time-consuming nature of GC-MS, we strove to find a more efficient method to quickly assess smoke exposure based on surface-enhanced Raman spectroscopy (SERS).

A Thermo Scientific Nicolet 6700 FT-IR / NXR FT-Raman spectrometer with a 785 nm laser was used to collect spectra. Raman spectra of volatile phenols were compared in synthetic wine. Silver nanoparticles were prepared in the lab and various silver and gold nanoparticles were obtained commercially. These nanoparticles were evaluated by SERS to enhance detection sensitivity. It was found that the commercially prepared gold nanoparticle strips can significantly enhance the absorption of guaiacol at 576 cm^{-1}. Using gold nanoparticle strip SERS, guaiacol can be detected at 100 µg/L in synthetic wine. Further research is being conducted on authentic wines.

Funding Support: American Vineyard Foundation and Oregon Wine Board

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Effect of Biofungicides on Grape Quality, Composition, Fermentation, and Sensory Characteristics of Wines from California

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Synthetic fungicides have demonstrated adverse environmental effects on finished wine attributes. Alternatives such as biofungicides employ organisms to control pathogens. The purpose of this study is to assess the effects of biofungicides on grape and wine chemistry, fermentation, and wine sensory characteristics. Four separate grape crops treated with biofungicides were vinified: Pinot noir and Chardonnay from the Central Coast, and Carignan and Chardonnay from the San Joaquin Valley. Treatments consisted of three different biofungicides: two bacterial strains (*Bacillus subtilis* strain QST 713 and *Streptomyces lydicus* strain WYEC 108) and an extract of *Reynoutria sachalinensis*. Two controls consisted of synthetic fungicide applications and no fungicide. All treatments were carried out in quadruplicate. Data has been collected on fermentation and basic grape and wine chemical parameters (total soluble solids [TSS], pH, tiratable acidity, and ethanol). Preliminary results for the Central Coast indicate significant differences in TSS compared to untreated controls, while no significant differences were found in grape and wine chemical parameters for the San Joaquin trials. Preliminary sensory trials show differences in color and aromatic intensity between treatments. Further analyses will be conducted on the wine color, phenolic content using high-performance liquid chromatography, and sensory characteristics using descriptive sensory analysis.

Funding Support: CDFA, CSU-ARI

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Effect of Soil and Foliar Nitrogen Applications on Syrah Fruit and Wine Tannin Concentration and Composition

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Syrah fruit and wines from a Columbia Valley vineyard trial in Washington State were evaluated. Nitrogen was applied at four different rates: to early-season soil at 0, 22.5, 45, and 90 kg N/ha and at veraison foliar at 15 kg N/ha; these treatments were denoted as control, low, medium, and high N and urea. Treatments were applied to three different vine rows in a randomized block design in 2021 to 2023. Fifteen wines were made for each vintage (five treatments, three replicates). Fruit and wine anthocyanins, grape seed, and skin tannins were analyzed for concentration (2021 to 2023) using the Adams-Harbertson assay and composition using phloroglucinolysis (2022-2023). Phenolics were measured daily during fermentation and during aging. At harvest, N-containing compounds including ammonia, yeast available nitrogen (YAN), and protein were measured. YAN and ammonia increased in the high N and urea treatments, with some exceptions. No significant differences were found in seed tannin concentrations for the three vintages, and no clear pattern was observed for skin tannins or anthocyanins. However, wine tannin concentrations were significantly greater in the control than in the high-N application treatment in the 2021 vintage, and both the control and low treatments were significantly greater than the high and urea treatments in the 2022 and 2023 vintages. Protein concentration had the opposite trend, with higher juice protein concentrations in the medium, high, and urea treatments, although the measures varied considerably. The phloroglucinolysis results show no difference in fruit tannin size or composition; however, the high N and urea treatment wines had significantly smaller average polymer size. The results show that the vineyard N applications increased ammonia, YAN, and juice protein, but the increased protein may be responsible for the reduced tannin concentration and size observed in the high N and urea treatments.

Funding Support: USDA-NIFA SCRI, Washington Wine Commission, Washington State Grape and Wine Research Program, Ste. Micelle Wine Estates (in-kind)

Chemical and Redox Potential Profiles of Assyrtiko Wines from California Fermented With and Without Solids

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Assyrtiko grapes (20.1 Brix, pH 3.19, titratable acidity 8.5 g/L, liquid:solid ratio 9:1) from the Paso Robles AVA of California were made into wine following traditional white wine fermentation carried out either at cold (between 15 and 17°C, average 15.8°C; WF-C) or warm fermentation temperatures (between 19 and 26°C, average 20.7°C; WF-W) or in an orange wine style, i.e. in contact with fermentation solids (skins and seeds), also at cold (SF-C) or warm temperatures (SF-W). Final alcohol levels were lower in skin-fermented (~11% ABV) than in white-fermented wines (~11.9% ABV). The redox potential oscillated between -100 and -20 mV during the first four days of skin contact time, then reached up to 100 mV at the end of skin contact time in skin-fermented wines. Conversely, white fermentations showed consistent redox potentials in the -100 to -120 mV range, and as low as -160 mV. There was more

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acetaldehyde in wines that fermented cold, and were generally higher (36 mg/L) than acetaldehyde levels normally found in finished red wines. Tannins and total phenolics averaged 10 and 82 mg/L in WF wines, respectively, and 308 and 885 mg/L in SK wines, respectively, and were slightly higher in wines fermented warmer. As expected, wine color was 87% higher in SF wines. Assyrtiko wines made with skin fermentation had tannin and total phenolic profiles similar to those observed in Central Coast AVA Pinot noirs, although they did not produce polymeric pigments. This is the first time, to our knowledge, that the phenolic profile of skin-fermented Assyrtiko wines, a staple variety from Santorini (Greece), has been described. This work is part of a larger project aiming at assessing the winemaking potential of emerging varieties of Mediterranean origin in the Central Coast of CA.

Funding Support: Richard Lauchland (Duas Terras Vineyards, Paso Robles, California).

Influence of Sodium and Calcium-based Bentonite Dosage Rates on Alcoholic Fermentation Kinetics in Chardonnay Wines

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Bentonite is commonly used in production of white wines to clarify must before alcoholic fermentation and to remove unstable proteins responsible for turbidity. However, it can also remove nitrogen compounds that affect fermentation kinetics and the development of wine aromas. We investigated how sodium-based and sodium-calcium-based bentonite dosage rates affect fermentation kinetics, yeast assimilable nitrogen (YAN), and standard chemical composition in Chardonnay wines from the Edna Valley AVA. Bentonite dosage rates of 0, 0.24, 0.48, and 0.72 g/L were applied to 60 gallons of must with four replicates per treatment at a commercial winery. Our initial findings show that regardless of dosage or bentonite type, wines treated with bentonite experienced a longer lag phase and took approximately one additional day to reach 0 Brix compared to the control wine. In sodium-based bentonite treatments, the 0.72g/L dosage significantly decreased YAN levels by 15 mg/L compared to the control, while with calcium-based bentonite, there were no significant differences observed in YAN concentrations across dosage rates. Future analysis, including gas chromatography-mass spectrometry, color, and phenolic compounds, will provide additional insights into the effects of bentonite treatment on Chardonnay wine quality.

Funding Support: Center of Effort Winery

Effects of Sulfur Nutrient Additions on Thiol Concentration in Pinot noir

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The wine industry is continuously threatened by the presence of wildfire smoke. Grapes exposed to smoke in the vineyard result in an ashy flavor in the finished wine. This flavor negatively affects wine quality, and in many cases, smoke-affected grapes are not made into wine. This can result in significant economic losses for the wine industry. Smoke-related compounds, thiophenols and volatile phenols, in combination cause the ashy flavor associated with grape smoke exposure. The formation of varietal thiols, important aroma compounds in Sauvignon blanc, has been researched extensively. An increase in sulfur-containing nutrients increases production of varietal

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thiols in Sauvignon blanc. We investigated whether adding sulfur-containing nutrients increases the concentration of smoke-related thiophenols during fermentation. In this study, smoke-exposed Pinot noir grapes from Oregon were made into wine using microferments. Different nutrient additions were added prior to yeast inoculation. Treatments included glutathione, glutastar, and diammonium phosphate (DAP). There were two controls: smoke-affected grapes with no nutrient additions and non-smoke-affected grapes with no nutrient additions. Free phenols were analyzed using GC-MS/MS; phenol-glycosides and thiols were analyzed using LC-MS/MS. The nutrient additions had no effect on the fermentation process, as all wines went through fermentation successfully. Results from this project are significant as they will provide another technique for winemakers to alleviate the effects of smoke exposure through varying nutrient additions during fermentation. Altering nutrients is an easy and inexpensive way for winemakers to manage this environmental issue.

Funding Support: USDA-ARS

Characteristic Extraction of Phenol Compounds in Koshu (*Vitis vinifera* cv.) Wine during Maceration

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Koshu is an indigenous grape variety that has been grown in Japan for over a thousand years. Recent research showed that it has 70% *Vitis vinifera* genes. It is the most-cultivated variety for winemaking in Japan.

The variety is mainly used to produce white wines, including a skin-contact fermented white wine which is fermented with seeds and skin. There little known about the extraction of phenol compounds during fermentation and maceration of Koshu wine. Thus, the objective of this study is to reveal the character of skin-contact fermented white wine by examining its phenolic compounds.

In this study, Koshu was compared with other *V. vinifera* varieties, Sauvignon blanc (SB) and Merlot (MN). Fruit tissues were separeted into pulp, seed, and skin and soaked separately in model wine solution (12% ethanol, 3000 mg/L tartaric acid, pH 3.2) for 14 days to determine extraction of phenolic compounds. The results showed that proanthocyanidin was extracted from SB and MN seeds and its content increased during soaking. Proanthocyanidin was not extracted from Koshu seeds, despite the presence of proanthocyanidin. The three varieties were fermented with seeds and skin during 28 days to study the behavior of extraction of phenolic compounds during fermentation and maceration. The results showed that the content of proanthocyanidin in Koshu wine decreased dramatically during maceration. In contrast, that of SB and MN wine was stable or increased during maceration.

These new findings on the unique characteristics of proanthocyanidin in Koshu grape show the diversity of character in *Vitis* and contribute to the control of wine taste.

Funding Support: Kirin Holdings, Company, Limited

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Exploring Dipeptides and Oligopeptides in Wine by UHPLC–HRMS

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Many dipeptides and oligopeptides identified in fermented foods and beverages have various taste-active or taste-modulating activities that encompass all five basic taste modes plus kokumi sensation. The presence of peptides in wine and their potential bioactivities and contributions to wine oxidative stability have been examined previously. However, the diversity of endogenous small peptides in wines remains unexplored. This study describes ultra high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) analysis of dipeptides and polypeptides in a range of commercial wines. Four Chardonnay wines produced at a commercial scale from vintage 2022 were screened and quantified for 400 common dipeptides by a previously developed chemical isotope labeling UHPLC Orbitrap HRMS approach. A total of 285 dipeptides were identified and quantified across all four wines at varied abundances. Dipeptide data was evaluated by principal component analysis, which showed clear and significant discrimination of wines. The most abundant dipeptides were in the wine that underwent malolactic fermentation, sur lie aging, and was aged in an oak barrel. No obvious correlation between individual amino acid and dipeptide was noted. In addition, untargeted peptidomic profiling based on UHPLC Orbitrap HRMS was developed and applied to a larger set of wines, including both commercial and experimental wines, for endogenous oligopeptides. Data-dependent acquisition HRMS spectra of non-tryptic wine samples showed the presence of large numbers of oligopeptides in wines. The peptide identifications were searched against literature peptide databases to screen sensory-relevant peptides. Salt-enhancing, bitter, sour, astringent, and kokumi peptides were found in the selected wines. The results presented in this study offer new insights on wine composition and pave the way for future study investigating the quality and sensory relevance of wine peptides.

Funding Support: E & J Gallo Winery

Sauvignon blanc Wine Protein Stability as Affected by pH Adjustment and Timing of Bentonite Addition

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The effects of pH adjustments on Sauvignon blanc wine protein haze formation and the required amount of bentonite were investigated at both microscale and commercial scale, using identical juice. Additionally, three different timings for bentonite addition were examined during the pH adjustment trial: before, during, and after fermentation. The study used the hot and cold test to determine the bentonite requirement for protein stabilization. Wine proteins were analyzed using various techniques, including a modified Coomassie brilliant blue assay, lithium dodecyl sulfate polyacrylamide gel electrophoresis, and sodium dodecyl sulfate capillary gel electrophoresis. Findings revealed that lower juice pH levels (2.80 and 3.00) resulted in sluggish fermentation, while the presence or absence of bentonite during fermentation did not significantly alter fermentation kinetics at any pH level. Bentonite remaining in contact with ferment improved fermentation completion for the sluggish ferment (pH 2.80). Wines with lower pH exhibited reduced wine protein

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content and enhanced protein adsorption efficiency of bentonite fining, requiring lower bentonite dosages for stability. Bentonite addition during fermentation proved most effective in protein removal, while fining after fermentation required the least overall bentonite dosage. Although different fermentation scales minimally affected wine protein contents, they did not alter molecular weight (MW) profiles. The protein contents and MW profiles in stabilized wines were influenced by the original juice pH, displaying more complex patterns in high-pH juice.

Funding Support: Lincoln University

How Many Strains of *Brettanomyces* are there? Integrating Global and Local Data Sets for Comprehensive Population Genomics

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Brettanomyces, best known for its significant economic impact upon the wine industry through spoilage of premium red wine, contributes to a range of fermentation processes worldwide. Previous studies have explored the genetic diversity of *Brettanomyces* isolates from wine and other sources, observing significant differences in properties with implications for wine spoilage potential. A notable example is sulfite tolerance, with tolerant isolates exhibiting similar genetic profiles found in several winemaking regions. Yet, these analyses remain fragmented due to their focus on different geographic origins and isolation sources, making it difficult to establish a clear picture of how many different populations, or strains, of *Brettanomyces* exist in association with a given source, such as wine.

Our research seeks to fill this gap by integrating several existing whole-genome sequence public data sets comprising 221 *Brettanomyces* isolates with data from our laboratory that spans a further 346 isolates. Our data also provides the first insights into *Brettanomyces* from Oregon's Willamette Valley. The biogeographic patterns of *Brettanomyces* reflect a combination of natural dispersion, human-mediated transportation, and ecological adaptation. Through integrating this data, we seek to establish a definitive resource for *Brettanomyces* population genomics, shedding light on its varied effects on wine spoilage by geographic origin and environment. Importantly, we will use this resource to answer the question of how many strains of *Brettanomyces* there are, and how many are associated with wine.

Funding Support: USDA Agricultural Research Foundation Oregon Wine Board New Zealand Wine

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Enzymatic Degradation of Undesirable Phenols in Wines Contaminated by *Brettanomyces* or Smoke Taint

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This is the first year of a multi-year project to discover and design enzymes with *exclusive* degradation of 4-ethylguaiacol (4EG) and 4-ethylphenol (4EP), the main sensory-active components in wine contaminated with *Brettanomyces* and responsible for a portion of the 'taint' phenols found in smoke-tainted wine (Figure 1). A library of 46 laccase enzymes were produced and tested in different buffered environments for activities on 4EG and 4EP, with multiple laccases degrading both phenols. All laccases were tested further in a model wine environment with 13% ethanol and tartaric acid, at pH 3.5. Multiple laccases quickly and completely

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degraded 4EG, along with mild activity on 4EP. Sequence analysis of the 4EG- and 4EP-active enzymes revealed two distinct clusters, each with a unique laccase that can degrade 100% of 4EG and 30% of 4EP in industrially relevant times, matrices, and concentrations. 4EP and 4EG docking interactions generated via computational modeling revealed the top-degrading laccases all display open active sites and likely contribute to activity on other, non-target, wine phenols, thereby reducing possible selective degradation of 4EG and 4EP. Currently, novel enzyme structures are being generated computationally to address this deficiency using open-source AI-tools, including RFDiffusion and LigandMPNN, with unprecedented abilities to design proteins and model their structures. The next phase of this project will use the knowledge of how laccases degrade 4EG and 4EP to create additional enzymes capable of fully, and exclusively, degrading the remaining smoke taint marker compounds including guaiacol, 4-methylguaiacol, *o*-, *p*-, and *m*-cresol, and syringol. The larger goal of this effort is to create a suite of novel enzymes that beneficially modulate wine flavor.

Funding Support: American Vineyard Foundation

Using In-Source LC-MS/MS Fragmentation to Fingerprint Tannin Structural Diversity and Protein Precipitation

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Procyanidins (PCs), or condensed tannins, significantly influence the mouthfeel and stability of red wine and polyphenol-rich foods. However, current analytical methods sometimes fail to correlate perception of astringency or predict tannin retention properly. This may be partly because common analytical methods oversimplify phenolics or tannins into one value (e.g., total concentration), not accounting for their diverse structures and sizes. Additionally, precipitation by methylcellulose (MC) or bovine serum albumin (BSA) employs reagents that only partially resemble endogenous grape proteins or human saliva (HS). In relation to perception, HS is more appropriate for studying complexation with tannins, as it contains the actual proteins and quantities inducing in-mouth precipitation, rather than analogs. A rapid ultra-performance liquid chromatography-tandem mass spectrometry-based method, coupled with in-source fragmentation, was used to quantitatively analyze and fingerprint a wide array of PC structures in wine after precipitation with MC, BSA, and HS. PCs were first fragmented using three different cone voltages (CVs) in the ESI interface, then further fragmented in the collision cell to enable their selective detection using optimized MRMs, which are finally used in a model developed for sample characterization and creation of a tannin "fingerprint". The "fingerprint" is composed of a variety of MRM transitions and their corresponding ratios for PCs across CVs, resulting in high-dimensional vectors for each PC rather than a single value, as seen in previous methods. Comparing "fingerprints" of PC standards to samples allows accurate identification and quantification of unknown PCs. Accuracy of the model was expressed as root mean squared error ranging from 0.0001 to 0.1475. Precision, expressed as relative standard deviation, was <0.82%. Within a one to five DP range of the "fingerprint", HS removes more small oligomers and monomers than BSA and MC, suggesting potential underestimation of low molecular weight tannin effect on astringency by BSA and MC.

Funding Support: None

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Effect of Yeast Strain Selection on the Concentration of Smoke-Derived Volatile Phenols and Thiophenols in Wine

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Wildfire and controlled burn events pose an economic threat to the global wine industry. Smoke-derived volatile phenols (VPs) exist as free and bound glycosidic precursors that are released during fermentation-driven enzymatic hydrolysis, but the role that yeast might play in thiophenol production has not been studied. Free VPs and thiophenols of smoke-affected wines have been shown to cause perception of undesirable attributes such as meaty, smoky, medicinal, and ashy aromas and flavors. Here we studied the effect of commercially available yeast strains on the concentrations of known smoke-derived VP and thiophenol markers. Grapevines (*Vitis vinifera* cv. Merlot) were experimentally exposed to smoke in a semi-controlled environment. Smoke-exposed and non-exposed grapes were fermented using 12 commercial yeast strains over two vintages. Gas chromatography-mass spectrometry confirmed the increased concentrations of VPs in wines from smoke-affected fruit compared to the control wines, but there were no differences in concentrations of VPs among the tested yeast strains. Investigation of thiophenols using high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry revealed differences between control and smoke wines in the relative peak area for thioanisole and thiophenol, but not for thiocresols or thioguaiacol. There were, however, differences among yeast strains for total thiocresols (*m*-, *o*-, and *p*-isomers) and for thioguaiacol. Our study suggests that commercial yeast strains play a role in the release of VPs and the concentration of thiophenols.

Funding Support: USDA, Washington Wine Commission, Jackson Family Wines.

Advancements in Rapid Smoke Taint Evaluation with Absorbance-Transmission and Fluorescence Excitation-Emission Matrix

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The urgent need for a rapid and cost-effective analytical method to assess the risk of smoke taint in wine emerged during the 2020 growing season in North America. Extensive wildfires along the West Coast inundated analytical laboratories with samples, creating a substantial backlog that extended delivery of customer results by over a month. This unmanageable timeframe resulted in significant economic losses for many growers and wine producers. Currently in development is a novel analytical model focusing on rapid and accurate predictive quantification of volatile phenols using absorbance-transmission and excitation-emission matrix (A-TEEM). Experimentation includes investigating multiple sample dilution levels to enhance the traceability of absorbance and fluorescence spectral signatures and of Beer-Lambert linearity. The development of the predictive model involves multiblock data analysis using a combination of univariate, multivariate, and machine-learning techniques to regress A-TEEM's spectral data against tandem mass spectrometry reference chemistry (GC-MS/MS). Statistical methods such as gray classical least squares, parallel factor analysis, partial least squares (PLS), PLS regression, and extreme gradient boosting are under evaluation for model optimization. The model

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will be developed based on the analysis of linear dilutions of severely smoke-affected wines with their non-smoke-affects counterparts, and its robustness will be enhanced by incorporating 10 different varietals harvested from multiple Northern California vineyards and produced over several vintages. Alongside the predictive quantification of volatile phenols, efforts are underway to create a comprehensive smoke taint index. This index seeks to establish a connection between the predictive model and sensory perception by implementing modified descriptive analyses. The resulting predictive sensory and quantitative models will offer invaluable risk assessment tools for the wine industry.

Funding Support: California Department of Food and Agriculture Specialty Crop Multi-State Program

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Determining the Spoilage Potential of *Brettanomyces* Strains Isolated from Oregon Vineyards and Cellars

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Oregon wine producers can face economic losses from wine spoilage from *Brettanomyces bruxellensis* infections. Despite precautions to mitigate infections by *B. bruxellensis*, such infections still occur. Variation in mitigation efficacy may be due to the emergence of sulfite-tolerant strains of *B. bruxellensis* in the wine production facility, as well as differences in quantity and strain of *B. bruxellensis* on the grapes entering the winery. Limited knowledge exists regarding whether known or novel sulfite-tolerant strains are present in OR wine facilities. Furthermore, the extent to which *B. bruxellensis* exists in vineyards is not well characterized due to the difficulty of isolating this yeast when more-abundant background yeast are present. This study seeks to address the limited understanding of the spoilage potential of *B. bruxellensis* strains found in OR cellars and vineyards.

We collected 100 Pinot noir cluster samples during the 2023 harvest from five different OR vineyards and will use an additional 105 cluster samples cryopreserved from the 2022 harvest. Collected vineyard samples will be grown in a selective enrichment medium that is currently being optimized to preferentially grow *B. bruxellensis* and limit background yeast growth. We also collected 52 wine samples and 79 environmental swabs representing seven OR cellars. Any identified *B. bruxellensis* isolates from the vineyard and cellar will be whole-genome sequenced and evaluated with sequence alignment to determine how related OR strains are to known strains outside of OR, particularly sulfite-tolerant strains. A preliminary enrichment formulation trial using cellobiose as the sole carbon source shows promising inhibition of background yeast, while not impeding growth of most *B. bruxellensis* isolates. The outcomes of this study will clarify the spoilage capacity of *B. bruxellensis* in OR wineries and vineyards, supporting wine producers to make informed decisions on how to best protect their wine from infections.

Funding Support: Oregon Wine Board

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Impact of Yeast Assimilable Nitrogen and Sugar on Sequential Fermentation with *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*

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We investigated the application of a low ethanol-producing yeast during co-fermentation with *Saccharomyces cerevisiae* to lower alcohol concentrations in wine. Specifically, this study investigated the complex relationships between yeast assimilable nitrogen (YAN) and sugar concentrations on *Metschnikowia pulcherrima* in sequential fermentation. A two three factorial was designed, with YAN (40 mg N/L or 280 mg N/L) and soluble solids (24, 27, or 30 Brix) as variables, by modification of synthetic grape juice media (SGJM). SGJM was fermented by either *S. cerevisiae* alone, or by *M. pulcherrima* and *S. cerevisiae*, the latter inoculated on day 4. Fermentations inoculated with *M. pulcherrima* reached similar maximum populations (ffi107 CFU/mL), except for those containing 40 mg N/L at 30 Brix, where populations were only 106 CFU/mL. In general, *M. pulcherrima* exhibited an extended lag phase before log growth in media containing 30 Brix, regardless of initial YAN concentrations. All fermentations with 40 mg N/L progressed slower than fermentations with 280 mg N/L. Low YAN fermentations only reached dryness (completion) at 24 Brix, which occurred six days after high-YAN fermentations at the same sugar level. SGJM fermented with *M. pulcherrima* and *S. cerevisiae* had lower glycerol concentrations than single-inoculation fermentations in 27 and 30 Brix treatments. Contrary to previous studies, ethanol concentrations were not affected by the presence of *M. pulcherrima*, except for the treatment containing 40 mg N/L with 24 Brix, which resulted in 0.7% v/v reduction.

Funding Support: Washington State Grape and Wine Research Program

Dissolved Oxygen Removal in Wines by Membrane Contactor and Nitrogen Sparging

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Many enological practices can result in excess dissolved oxygen in wine, leading to sensory and chromatic defects over time. Effective management of O_2 levels before bottling is crucial. This study employed nitrogen sparging and a polypropylene hollow fiber membrane contactor apparatus to regulate dissolved O_2 in wines derived from two grape varieties, Aglianico and Greco. Dissolved O_2 levels were monitored during treatments, with all treated wines achieving levels <1 mg/L. Phenolic compounds and acetaldehyde were assessed using high-performance liquid chromatography, volatile compounds were determined via gas chromatography-mass spectrometry, and chromatic characteristics were evaluated using spectrophotometric methods. Analyses were conducted immediately after treatment and after six months of aging under controlled O_2 conditions. Following aging, both Aglianico and Greco treated wines had less acetaldehyde content and higher levels of free and total SO_2 than control wines. However, no significant differences were detected between N sparging and membrane contactor treatment for the other parameters evaluated.

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Characterization of Polyphenol Elongation Induced by Exogenous Acetaldehyde

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Small amounts of oxygen are consumed during red wine aging, causing a cascade of reactions resulting in polyphenol elongation. The actual elongation is induced by acetaldehyde, an oxidation product of ethanol, creating ethylidene bridges between polyphenols. Elongation decreases bitterness and astringency while increasing color stability. Natural ingress of O_2 can cause other unwanted reactions and off-flavors depending on the stability of the wine and, in extreme cases, may even facilitate the growth of *Acetobacter* sp. It has been previously demonstrated that the direct addition of acetaldehyde can be added postfermentation to induce elongation, while avoiding excess dissolved O_2. To further study this reaction and the products produced, exogenous acetaldehyde was added to model wine (12.5% EtOH, 250 mg/L catechin, pH 3.5) at 0, 50, and 250 mg/L, at two temperatures (4° and 35°C) with and without SO_2. Solutions were sampled over 21 days, monitored using a liquid chromatography with tandem mass spectrometry (LC-MS/MS)-based method developed with multiple-reaction monitoring for ethylidene bridged catechins. Increasing temperature and acetaldehyde concentration increased the rate at which products were formed. Sequential addition of acetaldehyde instead of one large addition did not result in an ultimate difference in elongation products. Additionally, the addition of SO_2 at 250 mg/L did not fully inhibit elongation, indicating this reaction can proceed even in the presence of free SO_2, though at a decreased rate. To further characterize products formed, isotopically labeled acetaldehyde-d4 was reacted with catechin. Using a metabolomics workflow, ions produced from adding acetaldehyde and acetaldehyde-d4 were identified, showing the production of many more products than had been identified previously. Further investigations of this type were conducted with a mixture of catechin, procyanidin B2, and malvidin-3-*O*-glucoside. Acetaldehyde was added to Cabernet Sauvignon wine and the same ions were observed through LC-MS/MS. Ultimately, this research could give a new tool for winemakers to enhance their wine.

Funding Support: Pennsylvania Liqour Control Board

Assessing Color and Phenolics of Wines Produced from Co-fermentation of *Vitis rotundifolia* and *Vitis vinifera* Grapes

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While *Vitis vinifera* grapes are used predominantly for wine production, muscadine grapes (*Vitis rotundifolia* Michx.), native to the southeast United States, are also used but can have issues with color and phenolic stability. Cofermentation, two or more grape varieties fermented together, can enhance wine phenolic stability. Furthermore, sulfur dioxide (SO_2) additions in wines can affect color. In 2023, Noble (*Vitis muscadinia*) and Merlot (*V. vinifera*) grapes were harvested from commercial vineyards, randomized into five cofermentation treatments in duplicate (100% Noble; 75% Noble + 25% Merlot; 50% Noble + 50% Merlot; 25% Noble + 75% Merlot; and 100% Merlot), processed, and fermented. At bottling, SO_2 was added to each cofermentation treatment at different molecular levels (0.0, 0.8 and 1.5 mg/L). At bottling, wine pH (3.24 to 3.67), titratable acidity (0.60 to 0.80%), free SO_2 (18.53 to 42.43 mg/L), and ethanol (10.35 to 13.64%) varied for each cofermentation

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treatment. The cofermentation SO_2 interaction was significant for L*; 100% Noble without SO_2 had the lowest L* and 100% Merlot with 1.5 mg/L SO_2 had the highest. As Noble increased in each cofermentation treatment, red color, brown color, color density, total phenolics, and total anthocyanins increased. Wines without SO_2 had more red and brown color and greater color density than wines with 0.8 or 1.5 mg/L SO_2. Wines with 100% Noble had the highest total phenolics (2037 mg gallic acid/L) and total anthocyanins (1712 mg/L) compared to 100% Merlot (884 mg gallic acid/L and 296 mg/L, respectively). At bottling, wines with ≥25% Noble had higher color and phenolic content than 100% Merlot wines. Furthermore, SO_2 had more effect on L* in cofermentation treatments with more Merlot than Noble. Blends with more Merlot had more SO_2 bleaching, with lower phenolic and color content at bottling, while wines cofermented primarily with Noble grapes had darker color and more phenolics at bottling.

Funding Support: Southern Region Small Fruit Consortium Grant

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Genome-Wide Association Study of Anthocyanins and Phenolics in a Cold Climate Winegrape (*Vitis* spp.) Population

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To ensure superior wine quality and enhanced health benefits, it is desirable to maintain stability of anthocyanins and total phenolics in winegrapes. These compounds not only impart rich color and robust flavor to wine, but also include antioxidants that are beneficial to human health. However, ensuring their stability poses a significant challenge in North Dakota's harsh and variable climate. Deciphering genetic control of these compounds is crucial to influence the astringency, color, and mouthfeel of the wine. An incomplete diallel population of 1064 individuals was used to examine the genetic basis of key compounds for total phenolic and anthocyanin composition that affect both health benefits and sensory attributes of wine. To understand the genetic variation responsible for these traits, a genome-wide association study (GWAS) was conducted using 24,000 SNP markers. The GWAS revealed six significant SNP associations for anthocyanins on chromosomes 2, 5, 7, and 16 and three significant SNP associations for total phenolics on chromosomes 2, 6, and 11 over a period of two years. These findings will provide insights to understand the genetic factors affecting phenolic and flavonoid levels in winegrapes, offering a valuable resource for viticulture and enology, with potential applications for grapevine breeding for improved wine quality.

Funding Support: Specialty Grant

Correlating Volatile Phenol Concentrations in Cabernet Sauvignon Grapes to Unfavorable Sensory Characteristics in Wines

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Within California's wine industry, wildfire smoke poses a significant threat to vineyards by imparting negative sensory attributes to wines, termed "smoke taint." Hence, predicting the emergence of such tainted wines is crucial. This study evaluated the efficacy of a proof-of-concept approach to reproducibly expose

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grapes to smoke conditions. The long-term aim is to establish threshold levels of smoke-derived compounds in grapes that cause negative sensory attributes in the final wines. Around 250 kg of Cabernet Sauvignon grapes was harvested at 25 Brix from the Tyree vineyard at the University of California, Davis. Four experimental groups were exposed to various levels of smoke generated from red oak chips: control-exposure (CE, no smoke exposure), low exposure (LE, 0.5-hr exposure), medium exposure (ME, two-hr exposure), and high exposure (HE, four-hr exposure). Each experiment was conducted in an in-house constructed smoking chamber. Following smoke exposure, duplicate fermentations of each treatment were performed in stainless steel buckets. Volatile phenols (VPs) and their glycoconjugates (bound) were measured in grape and wine samples using gas chromatography-tandem mass spectrometry and liquid chromatography-tandem mass spectrometry, respectively. In addition, sensory differences in wines were determined utilizing descriptive analysis (DA). Free and bound VP concentrations in grapes and wines correlated positively with smoke exposure duration and intensity, as expected. Results indicated no significant sensory differences between LE and CE wines. ME samples were described as "medicinal" and significantly lower in fruity aroma than LE and CE samples. HE samples were rated even lower in fruity aromas and higher in "barbeque" and "ashy" aromas than all other treatments. Overall, ME wines had a significant sensory affect compared to CE wines, suggesting a potential range for future threshold experiments. The study was a successful proof-of-concept to develop threshold levels of free and bound VPs in grapes that will lead to unfavorable sensory attributes in the final wines.

Funding Support: USDA-ARS Funded Research

Sensory and Chemical Analysis of Cabernet Sauvignon Wines Made from Unripe, Ripe, and Overripe Grapes

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Cabernet Sauvignon grapes were harvested at three potential alcohols (11, 13.5, and 16% v/v, ABV) from a vineyard in Sunnyside, WA in 2018 and 2019. Alcohol was controlled for prefermentation by either dilution or chaptalization at each harvest. Basic wine chemistry was performed as well as untargeted solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) to identify major aroma compounds present. Targeted analysis was performed on various classes of wine aromatics (alcohols, esters, aldehydes, terpenes, and pyrazines) based on untargeted analysis. Descriptive analysis was performed on 2019 wines by nine panelists (four males). Ortho- and retro-nasal attributes were more significantly affected by ethanol than by harvest: seven ortho-nasal and eight retro-nasal attributes compared to one ortho-nasal and retro-nasal, respectively. Green bell pepper was significantly higher in the early-harvest and low-alcohol treatments, while dark fruit was higher in the late-harvest and high-alcohol treatments. Harvest affected astringency and bitterness, while alcohol affected all taste and mouthfeel attributes. Bitterness and astringency were highest in the late-harvest wines and sourness was highest in the low-alcohol wines. Principal component analysis of sensory data explained 89.5% of the data (75% on the x-axis) driven by alcohol. SPME-GC-MS showed that esters, alcohols, and aldehydes were significantly more abundant in higher-alcohol wines, while methoxypyrazines were lower in the early-harvest and higher-alcohol wines. Overall, the SPME-GC-MS results support the

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sensory data. Our results reinforce the growing multitude of data that shows that alcohol, rather than harvest date, controls flavor and aroma in wine.

Funding Support: Washington Wine Commission

Calcium Tartrate: New Assessment Method and Management of Instability

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The study addresses the critical challenge of calcium tartrate precipitation in white and rosé wines, an issue exacerbated by climate change. Due to the complexity of the processes involved in this phenomenon, there was no easily applied method available to define calcium tartrate instability. Research from Enartis introduces a groundbreaking evaluation method to obtain calcium tartrate stability, incorporating a comprehensive analysis of the effect of wine pH on tartaric acid dissociation and calcium tartrate formation. This new stability test for calcium tartrate provides more accurate prediction of potential instability.

The research also explores an innovative treatment strategy for wines exhibiting calcium instability. This involves the application of micronized calcium tartrate, proving its efficacy in reducing calcium concentration and enhancing wine stability. This approach to managing calcium tartrate stability was successful, confirming both the accuracy of the new test and the efficacy of micronized calcium tartrate in stabilizing solutions. This research marks a significant advancement in the wine industry's ability to predict and manage calcium tartrate instability, offering practical solutions for winemakers grappling with the implications of climate change on wine quality.

Funding Support: Enartis

Relevance of Nitrate Assimilation by *Brettanomyces bruxellensis* in Low-Nitrogen Environments

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Brettanomyces bruxellensis possesses the rare (among yeast) ability to assimilate nitrate, which confers sufficient advantage for it to outcompete *Saccharomyces cerevisiae* in fermentation processes enriched in nitrate, such as fermentation of sugarcane for bioethanol. When nitrate is abundant, *B. bruxellensis* can grow and ferment more rapidly under anaerobic conditions, through reversal of the Custers' effect and concurrent acetate production. Surprisingly, some *B. bruxellensis* strains cannot use nitrate due to structural gene deletions. Could this be due to lack of advantage in environments with low nitrate concentrations?

Genomes of 189 *B. bruxellensis* strains were analyzed for the presence/absence of nitrate assimilation genes, with 13 strains displaying at least partial deletion within the structural gene cluster. Previous studies showed loss-of-heterozygosity (LOH) events across this cluster, that may have some effect on phenotype. We found a high proportion of strains with LOH (68%) that varied with regard to the initiation of LOH from 0 to 10 kb upstream of the cluster. We are currently evaluating association of deletion and LOH events with different *B. bruxellensis* populations, and correlation with growth on nitrate.

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To evaluate the relevance of nitrate assimilation during fermentation in low-nitrate environments, one strain that displayed stronger growth on solid medium with nitrate was selected. Preliminary experiments in anaerobic environments show this strain used significantly more glucose ($p = 2^{e-16}$) and produced more acetic acid ($p = 2^{e-16}$) when grown on nitrate than ammonium, even at relatively low concentrations (0.042 g/L) of nitrogen.

Funding Support: Agricultural Research Foundation

Exploring the Synergistic Effects of *Lactobacillus plantarum* and *Oenococcus oeni* in Malolactic Fermentation

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The intricate dance of malolactic fermentation (MLF) in winemaking significantly influences wine quality, offering a playground for synergistic interactions between *Lactobacillus plantarum* (LP) and *Oenococcus oeni* (OO). This study examines the co-inoculation of LP and OO and their collaborative effect on MLF efficiency, bioactive peptide (bioP) production, and flavor development across different pH conditions. Using a refined experimental blend, we used lab-scale screenings and fermentation trials in must, providing insights into acetic acid production and the influence on wine sensory attributes. Preliminary findings suggest that strategic use of LP and OO not only expedites MLF completion, but also enhances a wine's organoleptic properties, establishing a compelling case for their combined use in modern enology. This abstract presents data from extensive trials, including modified inoculation strategies and the evaluation of acetic acid levels, illustrating the potential of these microbial partners to revolutionize winemaking practices.

Funding Support: Chr. Hansen

Natural Cork Classification Based on Oxygen Transmission Rate

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Managing oxygen exposure is a crucial and complex aspect of winemaking, affecting every stage from initial juice extraction to maturation. The amount of O^2 to which wine is exposed significantly influences its taste, aroma, and overall quality. In particular, white wines can suffer from premature oxidation during bottle aging, which deteriorates their sensory quality—a problem recognized since the 1990s, initially through changes in wine color. Research has since divided into two main paths: exploring how oxidation affects sensory perceptions and investigating the molecular mechanisms behind it.

The factors influencing oxidation in bottled wine include the wine's composition and antioxidant content, the presence of transition metals like iron and copper, the bottling process, variability in bottle necks, treatments at the cork-glass interface, and the O^2 transmission rate (OTR) through the cork. These factors are influenced by storage conditions such as time, temperature, pH, and bottle orientation.

To tackle the variability in OTR of natural cork stoppers, a new process has been introduced into production lines. This process uses advanced imaging and deep learning to analyze the internal structure of corks and correlate it with their OTR,

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effectively distinguishing corks with high OTR from the standard range. This allows for real-time sorting of corks during manufacturing, ensuring that only those with suitable OTR values are used, thereby enhancing the consistency and quality of wine by minimizing undesirable oxidation effects. This method has been validated through established OTR measurement techniques (UVAMOX, Spain).

Funding Support: Cork Supply Portugal, S.A

Tatumella ptyseos' Role in Early Degradation of Malic Acid in Grape Juice

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The 2022 and 2023 vintages presented *Vitis vinifera* juice samples with unexplained loss of malic acid prior to the onset of primary fermentation. These fresh juices, lacking malic acid, contained no detectable lactic acid bacteria (LAB) by common PCR and plating methods. Culture plating from these juices and 16S rDNA sequencing identified the predominant bacterium present as *Tatumella ptyseos*.

Prior research on vineyard microbiomes using NextGen sequencing indicates several *Tatumella* species are commonly associated with winegrapes. The presence of this rod-shaped, gram-negative, facultative, and anaerobic bacterium was associated with lowered acidity in the juice. The authors of the papers did not report on changes in specific acid components of the juice.

Initial experiments indicated the cause of lowered acidity associated with *T. ptyseos* in grape juice was due to rapid depletion of malic acid. Additional experiments were done to characterize the growth of *T. ptyseos* under standard winemaking conditions. These experiments evaluated growth rate, malic acid and sugar consumption, acid production, and tolerance to ethanol, temperature, and sulfur dioxide.

This is the first report confirming the ability of *T. ptyseos* to metabolize malic acid, resulting in a lower juice acidity.

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How Temperature and Nutrition Affect the Fermentation and Sensory Profile of Chardonnay

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Fermentative microorganisms are affected by the physiochemical characteristics of their environment. In winemaking, temperature and yeast nutrition play a major role in wine quality and sensory profile. They affect aroma production during fermentation and temperature also influences fermentation duration, which can modulate aroma content. While many studies focus on temperature and nitrogen effects, few examine the combination between these factors, including the type and timing of nutrient addition.

We studied the simultaneous effect of three factors on fermentation kinetics, aroma production during fermentation, and sensory profile: i. temperature of fermentation (T), ii. yeast assimilable nitrogen (YAN), iii. type and moment of nutrient addition (N), in a Burgundy Chardonnay wine.

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To do so, a Box-Behnken experimental design was used to determine 14 treatments to be studied, with a triplicate in the medium case. Studied temperatures were 12, 16, and 20°C, the ratio YAN/Sugar (mg N/g S) varied from 0.8, 1 to 1.2 in model N-deficient or excess musts, and nutrition was varied using organic or mineral N-rich nutrient, equally used at the pitching moment or at 30% fermentation. Fermentations took place in 20-L temperature-controlled vessels, on a Chardonnay must from Burgundy inoculated with 20 g/hL of yeast, and malolactic fermentation was blocked to avoid tasting bias.

Kinetics were monitored during fermentation and classical fermentative aromas were analyzed after verifying the implantation of the yeast in the must. Descriptive profile tasting was conducted by a panel of 10 well-trained professionals familiar with Chardonnay from Burgundy. This study presents a scientific exploration of fermentation management through temperature and nutrition regimes and illustrates the potential leverage of yeast to produce quality wines.

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Classical Least Squares Assay of Wine Colorants with Absorbance-Transmittance Excitation Emission Matrix (A-TEEM) Data

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Commercial additive concentrates (CAC) of Teinturier grapes with intensely colored skins and flesh, enriched in malvidin-3,5-diglucoside, are commonly used covertly to enhance color in finished wines from *Vitis vinifera* sp. grapes. The target CAC concentration is typically at least 0.2% by volume. This study investigates the detection of CAC with A-TEEM spectroscopy using a recently introduced, single-variable adjustment classical least squares (CLS) method known as Gray-CLS (Eigenvector Inc., Solo v9.3.1). The first-principles-based Gray CLS-results are compared to other multivariate regression methods, including partial least squares (PLSR), locally weighted regression (LWR), and extreme gradient boosting (XGBR). The experiment included several wines from different grape varieties spiked at varying concentrations with a commercially available CAC. A-TEEM measurements were collected under Beer-Lambert linear absorbance conditions at a constant temperature (20°C) using a standard solvent (50% EtOH, pH 2) and 0.45 micron filtration. The model data included both a calibration (cross validation set) of ~80% of the samples and an independent validation set comprising ~20%. The Gray-CLS model yielded a standard deviation (SD) of ~10% of the target CAC (0.2%) for the validation set by optimizing only the general least squares weighting variable of the CLS residuals. PLSR, XGBR, and LWR achieved similar or slightly lower SD values. However, these methods all required complex and potentially ambiguous optimization of multiple preprocessing variables of the spectral and concentration data blocks and other algorithm-tuning parameters, including the number of latent variables and/or principal components, among others. These methods, prone to under- or over-fitting, are thus potentially unreliable. We conclude the A-TEEM method can be an effective tool to quantify CAC using Gray-CLS to avoid issues with under- and over-fitting multivariate regression models, yielding results relevant to commercial wine quality evaluation.

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Enhancing Piquette Profiles with *Lachancea thermotolerans*

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Piquette is a low-alcohol beverage made primarily from leftover winemaking pomace and water. With historical roots and a recent resurgence, it meets modern consumer demands for lighter, sustainable beverages. Piquette fermentations often rely on ambient microorganisms, potentially resulting in unpredictable profiles. In this study, we tested the lactic acid-producing yeast *Lachancea thermotolerans* (Zymaflore Omega) in piquette production to address issues of low acidity/high pH. Sauvignon blanc pomace was sourced from a winery in Bordeaux after routine processing of grapes. Equal amounts of pomace were distributed into fermenters, followed by the addition of three different volumes of water: equivalent to, double, and half the volume of the pomace mass. For each water addition, two fermentation regimes were compared: inoculation with Omega (200 ppm) and uninoculated treatments. After five days, the samples were pressed off skins and subjected to physicochemical and sensory profiling. The analytical profiles varied based on water addition and fermentation regime. All samples fermented to dryness, with ethanol levels ranging from 3.9 to 6.7% ABV depending on the dilution. Samples with the highest ethanol levels had the highest pH (4.0) regardless of the inoculation regime. The lowest pH (3.8) was observed in the modality fermented with Omega, following double water addition. Malic acid was not detected in any samples, indicating complete MLF. However, Omega-treated samples had higher lactic acid levels due to partial conversion of sugars into lactate by *L. thermotolerans*. Conversely, uninoculated samples contained more acetic acid. The polyphenolic profiles and color (CIELAB) also differed between the samples. Inoculation of Omega resulted in higher scores of aroma intensity, thiols, balance, freshness, body, and overall impression, regardless of the water addition level. Together, these findings highlight the potential of harnessing microbial diversity to create alternative beverages with enhanced profiles, aligning with the principles of circular economy.

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New Kids on the Block: Novel Yeast Strains for Fresher Wine Profiles

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Trends continually evolve, and the wine sector is no exception. Consumers and winemakers alike are steering away from heavier wines in favor of lighter, fresher, and more aromatic profiles. This work focuses on selection and characterization of microbial strains suitable for achieving such profiles. Two novel *Saccharomyces cerevisiae* strains, Zymaflore Xarom and Klima, were developed using QTL-assisted breeding for enhanced aromatic intensity and lower ethanol yield, respectively. They were compared for their production of primary and secondary metabolites in a series of fermentation trials with variable temperature and grape juice composition. Unlike typical *S. cerevisiae* starters, both strains could either preserve or produce malic acid during alcoholic fermentation. The largest malic acid production (0.9 g/L) was observed in red wine fermented with Klima at lower fermentation temperature (21 versus 26°C) and increased sugar content (16 versus 15% vol. alc.). The pH/titratable acidity of wines were in line with the yeast-derived modulation of malic acid. In both strains, higher temperature and sugar content boosted glycerol production, irrespective of acetic acid formation. Moreover, the levels of volatile

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thiols systematically increased with higher white wine fermentation temperatures (21 versus 18°C). The two yeasts exhibited distinct ester profiles: Klima was associated with increased ethyl esters, which were more abundant at lower temperature. In comparison, Xarom favored production of acetate esters regardless of fermentation temperature. Sensory analysis validated specific metabolic fingerprints of each strain in different matrices, with temperature-derived modulation of fruity and fermentative notes. This study therefore highlights the efficacy of readily implementable solutions, such as yeast strains and fermentation temperature, to fine-tune freshness and differentiate wine styles.

Funding Support: Laffort

Bio-Acidification of Wines with the Yeast *Lachancea thermotolerans*

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Insufficient acidity in grapes is commonly corrected by adding tartaric acid during winemaking. An alternative approach involves bio-acidification with *Lachancea thermotolerans* (LT) via lactic acid production during fermentation. Our work first described the genetic and phenotypic diversity of ~200 LT strains, then tested the performance of their subset conjunction with *Saccharomyces cerevisiae* (SC). An LT strain with exceptional bio-acidifying properties was selected (Zymaflore Omega) and further characterized across a range of conditions. The follow-up study aimed to compare the profiles of bio-acidified LT wines and acid-adjusted SC wines, and to evaluate the use of LT wines as blending components. High sugar/pH Merlot was fermented with a sequential culture of LT and SC, and an SC monoculture. The aliquots of the SC control (4.0) were acidified with either tartaric or lactic acid to the pH of LT wine (3.6). The initial LT and SC wines were also blended in three proportions (1:3, 1:1, 3:1). Chemical analysis revealed major differences in various wine parameters (e.g., ethanol content, acidity, color, volatile compounds, amino acids). The compositional modulations were reflected in wine sensory profiles, confirmed via rate-all-that-apply evaluation by 30 wine experts. Sensory profiles of the bio-acidified LT wine and the lactic acid-adjusted SC wine were similar, contrasting with the tartaric acid-adjusted SC wine. Lactic acid-adjusted SC wine had enhanced red fruit flavor, with less hotness, bitterness, and body than the tartaric acid-adjusted wine. This was driven by differences in acidity perception, affected by titratable acidity (rather than pH) of wines. An inhibition of *Brettanomyces bruxellensis* was also observed in the bio-acidified LT wine and the lactic-acid adjusted SC wine. The profiles of blends were modulated depending on the proportion of the bio-acidified wine, highlighting the potential of this approach to adjust wine acidity and style.

Funding Support: Laffort

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New Microbial Tools to Face Climate Change

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During the past decades, drastic changes in grape must composition have occurred, leading to wines with higher ethanol content and lower acidity (Van Leeuwen et al. 2019). Both linked to climate change, these two features can be addressed concurrently using innovative techniques for microbial selection.

Many efforts focused on non-*Saccharomyces* yeast selection to lower the ethanol/sugars ratio during alcoholic fermentation. *Lachancea thermotolerans* is one of the most interesting species because it partially routes glucose and fructose to L-lactic acid, *de facto* lowering ethanol production during alcoholic fermentation while also acidifying the wines. Previous studies highlighted the importance of strain selection, as not all strains are alike (Hranilovic et al. 2018), leading to the selection of one strain, Zymaflore Omega. Further assays also showed that the enological environment and inoculation scenario drastically influence lactic acid production, as described here.

This strain must be associated with a *Saccharomyces cerevisiae* strain to complete alcoholic fermentation. Our latest selection program delivered a strain concentrating most genetic elements linked to malic acid preservation/production and lower ethanol/sugars yield, named Zymaflore Klima. The L-malic acid production of this strain depends on the initial malic acid content but can lead to 0.5 to 1 g/L produced in low-malic acid musts, while losing 0.2 to 0.3% vol ethanol.

Malolactic fermentation leads to decreases in acidity due to transformation of L-malic acid into L-lactic acid. However, this can be tempered using an *Oenococcus oeni* strain selected for low citric acid consumption, named Lactoenos Berry. Considering several assays, results show that titratable acidity can gain 0.5 g/L as tartaric equivalents compared to indigenous strains.

Together, these three carefully selected microorganisms lowered ethanol by 0.5% vol and increased TA by up to 2 g/L.

Funding Support: Biolaffort

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Comparative Analysis of Glutathione Production by Commercial Non-*Saccharomyces* Yeast Strains in Wine Fermentation

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L-γ-glutamyl-L-cystinyl-glycine, commonly known as glutathione (GSH), plays a crucial role as an antioxidant in wine, helping to mitigate the loss of volatile flavor compounds susceptible to oxidation, as well as preventing oxidative browning and aroma changes. In recent years, particular interest in using GSH in winemaking has developed and as a result, yeast derivatives rich in this compound have been marketed to maximize and preserve flavor expression. Non-*Saccharomyces* yeasts (NSY) have been associated with producing higher concentrations of GSH than *Saccharomyces cerevisiae*, yet data on the production of this compound across different species or strains is limited.

This work investigates the potential of four commercial NSY strains from three species—*Lachancea thermotolerans*, *Pichia kluyveri*, and *Torulaspora delbrueckii*—to increase the GSH concentration in the final wine. Assessments will be conducted in two musts designed to emulate conditions for red and white or rosé wine fermentations, respectively. The initial yeast inoculation will take place with NSY before *S. cerevisiae* is inoculated 48 hours later.

Preliminary findings suggest that the non-*Saccharomyces* yeast strains exhibit varied capabilities in enhancing GSH levels, potentially offering winemakers new tools to improve wine quality and stability through natural antioxidant mechanisms. Further research and trials will illuminate the practical implications of these findings for winemaking practices, particularly for flavor preservation and oxidative stability.

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Sparkling Wines and Effervescence: Effect of Different Molecules on Foam Quality

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Foam is among the most important quality parameters we can assess in sparkling wines. The pleasure and sensations perceived when drinking a sparkling wine, its drinkability or aromatic bouquet, are closely linked to the wine's effervescence and the quality of its bubbles. To understand what influences this, it is pertinent to begin by studying objective analytical parameters in base wines.

The aim of this work, carried out in collaboration with the Department of Oenology at the University of Padua, was to clarify the role of "amphipatic" molecules, compounds that play a positive, active role in foam quality. At the same time, other molecules such as bentonite were studied for their opposite effect.

The Mosalux instrument, designed by the Station Oenotechnique de Champagne (SOEC), was used to collect data on three qualitative parameters used to assess foam quality: HM (maximum height), HS (stability height), and TS (stability time). This method enabled us to compare the positive or negative effects of different molecules on the foam quality of sparkling wines, and therefore their overall quality.

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WinePlus: The Revolutionary Remote and Real-Time Wine Fermentation Process Monitoring System

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The wine industry is moving toward digitalization of traditional measuring techniques. Fermentation is the most important stage of winemaking, where the sugar is chemically transformed into alcohol. Constantly monitoring parameters and adjusting them via the addition of enological products, according to readings and determined nutrient requirements, is not only labor-intensive, time-consuming, and inefficient, but also has limited forecasting capabilities. This is a major issue, as the incidence of stuck and arrested fermentations may reach 20%. Tardy, reactive adjustments of the fermentation conditions result in wine losses of both quality and quantity. The high carbon dioxide and water losses have led to the wine industry having the third largest carbon footprint of any industry. These factors combined make more efficient, economically and environmentally sustainable solutions for monitoring fermentation desirable. This solution must be fully integrated and provide real-time values of parameters that are predictive during fermentation. Wineplus is a revolutionary, cost effective, scalable, IoT-based, integrated solution for real-time monitoring of fermentation, with forecasting and preventive capabilities, to achieve sustainable, cost-effective production of high-quality wine. It uses the principle of pressure sensing and advanced AI algorithms to measure density accurately. It also measures the liquid level for tracking operations and volume monitoring. By implementing this system, Wineplus has already improved the vinification process by reducing time consumption up to 73%, allowing for multi-cellar control. This solution has the potential to reduce by 20% the carbon and water footprint of vinification. Several case studies of tank and barrel fermentations will be presented.

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