2022 Grape Day
April 26, 2022 | Corvallis, Oregon
Research Projects Related to Vineyards and Wine

The
Oregon Wine Research Institute

2022 SYMPOSIUM PROCEEDINGS

Oregon State University

Program Schedule

8:30 AM – 9:00 AM	**Registration Check-In and Refreshments**
9:00 AM – 9:15 AM	**Welcome and Introductions** *Dr. Staci Simonich, Dean and Reub Long Professor, College of Agricultural Sciences, OSU*
9:15 AM – 9:45 AM	**Optimizing Irrigation Initiation in Oregon Vineyards** *Dr. Alexander (Alec) Levin, Assistant Professor (Viticulture), Department of Horticulture, OSU-Southern Oregon Research and Extension Center*
9:45 AM – 10:15 AM	**Gene Editing Technology in Grapevine** *Dr. Laurent Deluc, Associate Professor (Grape Genomics and Genetic Engineering), Department of Horticulture, OSU*
10:15 AM – 11:15 AM	**Interactive Poster Session** This session will provide you the opportunity to interact one-on-one with scientists and students conducting research at the OWRI. Posters will feature research findings that are in-progress for various research projects being conducted across a wide array of topics within viticulture, enology, and economics.
11:15 AM – 11:45 AM	**A Handy Tool: New Methods in Monitoring Grape Powdery Mildew** *Sarah Lowder, Graduate Research Assistant and PhD candidate, Department of Botany and Plant Pathology, OSU*
11:45 AM – 12:15 PM	**Free and Bound Volatile Phenols in Smoke-Exposed Wines** *Dr. Michael Qian, Professor (Food Chemistry), Department of Food Science and Technology, OSU*
12:15 PM – 1:00 PM	**Lunch**
1:00 PM – 1:30 PM	**Determining the Chemical Components that Cause Sensory Qualities in Wine** *Dr. Elizabeth Tomasino, Associate Professor (Enology), Department of Food Science and Technology, OSU*
1:30 PM – 2:00 PM	**UV-C Light for Grapevine Disease Management** *Alex Wong, Graduate Research Assistant and PhD candidate, Department of Botany and Plant Pathology, OSU*
2:00 PM – 2:30 PM	**Break/Poster Session**
2:30 PM – 3:00 PM	**Applied Viticulture Research to Address Climate Change** *Dr. Patty Skinkis, Professor and Viticulture Extension Specialist, Department of Horticulture, OSU*

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Enology and Sensory Chemistry

Nitrogen, Potassium, and Magnesium Impact in the Vineyard on Vine Productivity and Quality

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The impact of nitrogen (N), potassium (K), and magnesium (Mg) supply on vine productivity and fruit composition is being evaluated at three commercial sites in Oregon as part of a nationwide vineyard nutrition study. Three rates of soil-applied N (no N, low N-20 lb./acre, and high N-40 lb./acre) were established for Chardonnay. Four treatments, including soil applications of 0, ~200, and ~400 lb./acre K or foliar sprays using Metalosate-K® were applied in a low K Pinot noir vineyard. Three rates of foliar-applied Mg (no Mg, low Mg-3 lb./acre, and high Mg-6lb./acre) were included in a low Mg Pinot noir block. All the treatments began in 2021 and each experiment has a randomized block design with four replicates. In the N trial, the high N increased leaf blade N at bloom, petiole N at veraison, and must yeast assimilable nitrogen (YAN) levels compared to the no N Control. However, vine growth and yield were not yet altered by N supply in Chardonnay. For the K experiment in Pinot noir, vine K status, growth, yield, and must chemistry were not altered by applied K. However, the K concentration in pruning wood canes at dormancy was increased for treatments that received K over no K Control vines. The Mg applied to the foliage of Pinot noir also had no impact on vine productivity although leaf blade Mg increased by a high rate at veraison. Both the low and high rate of Mg sprays reduced the leaves' number displaying Mg deficiency symptoms late in the summer. Additionally, the severity of Mg deficiency symptoms on leaves was related to leaf blade Mg concentrations near harvest but was not related to petiole Mg levels. All three studies will continue over the next two years where we expect greater cumulative impacts of nutrient supply on the vines.

Funding: National Institute of Food and Agriculture

Tilling in a Dry Season Doesn't Always Impact Soil Moisture or Reduce Vine Water Stress in Oregon's Willamette Valley

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Vineyards in Oregon's Willamette Valley are predominantly planted to perennial grasses in alleyways for worker and equipment traction. Perennial grasses may also reduce vine vegetative vigor compared to tillage. Spring tillage is often implemented to conserve soil moisture in vineyards with shallow soils, young vineyards, or in dry seasons. Previous research in the region found that alleyway vegetation did not compete with vines for soil water. However, those studies were in volcanic soils. More work is needed to understand vineyard floor management practices in different soils, as soils differ in physical and chemical properties, water holding capacity, and nutrient availability. The effects of vineyard floor management practices (till and no-till) were evaluated in one vineyard with three soil types derived from sedimentary, volcanic, and glacial deposit parent materials. Spring tillage and no-till treatments were applied to alleyways in a randomized complete block design with five to ten field replicates in each soil type, within blocks of the same cultivar, rootstock, age, vine spacing, and training system. Vine growth, water stress, soil moisture, yield, vine nutrient status, and fruit composition were measured in 2021. Results from this study indicate that vineyard floor management did not affect leaf stomatal conductance, stem water potential, or under-vine soil water content. Vines in tilled treatments had higher leaf blade nitrogen concentrations and leaf greenness compared to no-till treatments in all soil types. This is likely due to an increase in soil nitrogen from the tilling of alleyway vegetation since there were no differences in soil moisture. These results indicate that vineyard floor management practices have variable and limited impacts on soil moisture or plant water stress regardless of soil type in a dry season. Further research will be needed to determine the long-term vine impacts that till and no-till have in different soil types.

Funding: Oregon Wine Board and Erath Family Foundation

Optimizing Irrigation Initiation in Oregon Vineyards

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The scion When to initiate irrigation is a critical annual management decision that has cascading effects on grapevine productivity and wine quality in the context of climate change. A multi-site trial was begun in 2021 to optimize irrigation initiation timing using midday stem water potential (ψ_{stem}) thresholds characterized as departures from non-stressed baseline ψ_{stem} values ($\Delta\psi_{stem}$). Plant material, vine and row spacing, and trellising systems were concomitant among sites, while vine age, soil type, and pruning systems varied. Five target $\Delta\psi_{stem}$ thresholds were arranged in an RCBD and replicated eight times at each site: 0.2, 0.4, 0.6, 0.8, and 1.0 MPa (T1, T2, T3, T4, and T5, respectively). When thresholds were reached, plots were irrigated weekly at 70% ET_c. Yield components and berry composition were quantified at harvest. To better generalize inferences across sites, data were analyzed by ANOVA using a mixed model including site as a random factor. Across sites, irrigation was initiated at $\Delta\psi_{stem}$ = 0.24, 0.50, 0.65, 0.93, and 0.98 MPa for T1, T2, T3, T4, and T5, respectively. Consistent significant negative linear trends were found for several key yield and berry composition variables. Yield decreased by 12.9, 15.9, 19.5, and 27.4% for T2, T3, T4, and T5, respectively, compared to T1 (p < 0.0001) across sites that were driven by similarly linear reductions in berry weight (p < 0.0001). Comparatively, berry composition varied little among treatments. Juice total soluble solids decreased linearly from T1 to T5 – though only ranged 0.9 Brix (p = 0.012). Because producers are paid by the ton, and contracts simply stipulate a target maturity level, first-year results suggest that there is no economic incentive to induce moderate water deficits before irrigation initiation, regardless of vineyard site. Subsequent years will further elucidate the carryover effects of delaying irrigation initiation on productivity over the long term.

Funding: Oregon Wine Board

Water Stress Response of Chardonnay and Pinot noir on Different Rootstocks

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Rootstocks are used in vineyards worldwide; however, there is renewed interest in rootstock suitability given the need for grapevine adaptation to changing environmental conditions, especially drought. Rootstock studies indicate variable performance based on regional climates and soil types. As Oregon experiences warmer seasons and changing precipitation patterns, growers are interested in exploring rootstocks that provide more drought tolerance than the commonly planted rootstocks, especially under dry-farmed conditions. We evaluated vine drought stress response and vine performance in a trial of mature (> 20-year-old) Pinot noir grafted onto nine rootstocks and Chardonnay vines grafted onto five rootstocks and own-rooted vines of each cultivar. The cultivar x rootstock combinations were planted in a randomized complete block design with five field replicates. The vines were managed with a weed-free strip undervine, permanent resident cover in alleyways, and dry-farmed for more than a decade. During 2021, water stress response was measured by leaf stomatal conductance and stem water potential from fruit set to veraison. Chardonnay grafted to Riparia Gloire was the most stressed with the lowest stem water potential and lowest stomatal conductance of all Chardonnay-rootstock combinations. Likewise, Riparia Gloire had the lowest stomatal conductance compared to other Pinot noir rootstock combinations. Drought tolerant rootstocks 1616, 1103 Paulsen and 140R grafted to Pinot noir had less water stress (higher stem water potential) compared to drought sensitive rootstocks. Rootstock had the greatest impact on dormant pruning weight, suggesting the impact of prolonged water stress on grapevines, with drought tolerant rootstocks having larger vine size than drought sensitive rootstocks, according to 3-year means. These results demonstrate that drought sensitive rootstocks experience more water stress and have less vegetative growth regardless of the cultivar. This work will continue into 2022.

Funding: Oregon Wine Board and Erath Family Foundation

Applied Viticulture Research to Address Climate Change

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Climate variability has raised grower awareness of the vulnerabilities faced by the wine grape industry. Growers are pressured to reduce environmental impact by avoiding or reducing herbicide use and embracing farming certifications such as organic, biodynamic, and regenerative agriculture. These factors are impacting vineyard performance with adopted management strategies, such as no till and no herbicide, leaving vineyards over-stressed for nutrients and/or water resources based on the current vigor-reducing rootstocks and dry-farmed conditions in Oregon's Willamette Valley. To address these concerns and to provide regionally important information for growers, research studies including rootstock and soil-focused vineyard trials, have been in place since 2020. We are evaluating >20-year-old Pinot noir vines grafted to 19 rootstocks. Results indicate that Oregon's standard rootstocks (3309C, 101-14 and Riparia Gloire) are under more water stress (stem water potential) in mid-late summer than 1103P and 1616. Rootstock primarily impacts vine size as measured by dormant pruning weight, while yield and fruit composition have fewer differences. Two research projects focus on vine performance in three different soil types (sedimentary, volcanic, and glacial deposits) under standard (no-till) and spring tillage. The main study evaluating soil water content under-vine and in alleyways at 18" and 36" depths revealed the least water stress and most vine growth in the glacial soils, and smaller vine size and more water stress (leaf water potential) was found in the sedimentary and volcanic soils. In 2021, a till/no till study was conducted to evaluate the water conservation potential of spring tillage practices in these soil types. Spring tillage had limited to no impact on soil moisture conservation or plant water stress during summer in any soil type. Together these studies are providing information that will help us design better vineyards and employ better practices under dry-farmed conditions in Oregon's Willamette Valley.

Funding: Oregon Wine Board and Erath Family Foundation

The Gene Editing Technology in Grapevine: A Focus on Transgene-Free Gene Editing

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Gene editing is a new technology tool using a Sequence-Specific Nuclease (SSN) that acts as "molecular scissors" to cut the DNA. The most common and versatile SSN used in the plant community is the CRISPR/Cas9 system, which contains a protein (Cas9) and an RNA molecule called single guide RNA (sgRNA). The protein Cas9 will cut the DNA while the sgRNA will guide the Cas9 protein to the targeted nucleic region. Plant DNA repair mechanisms will then "fix" the cut using two pathways, the Non-Homologous End Joining and the Homology Direct Repair pathways. While the first is prone to "errors", the second will adequately repair the cut. Plant scientists exploited both features, to develop what we could call conventional gene editing (imperfect repair) and precision gene editing (engineered repair). More versatile and precise than traditional tools of silencing and overexpression, gene editing can offer significant opportunities to create new varieties because it is seen as a plant breeding technology. There are two ways to perform gene editing in plants using transgenic and non-transgenic methods. The transgenic process will insert the gene ingredient and other selection markers in the genome of the model plant physically to conduct the editing. In sexually propagated crops, one can easily remove the "transgenic markers" in the progeny by selfing or backcrossing to obtain a transgene-free gene-edited plant material. The non-transgenic methods will deliver the gene-editing ingredient information to the cells that can regenerate into an individual plant as either a DNA molecule or a protein. Protoplasts or "naked cells" are often used as plant material. In grapevine, unfortunately, both ways designed to avoid the GMO label are challenging. We developed two projects related to these critical questions. The values and pitfalls of both approaches will be discussed, along with the significance and impacts on grapevine production.

Transgene-Free Gene-Editing in Grapevine for Powdery Mildew Resistance

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In the U.S., gene-edited crops are regarded as products of plant breeding if no foreign DNA is integrated into the genome (transgene-free) and face less regulatory hurdles. The conventional approach to editing plant material is via *Agrobacterium*-mediated transformation, which leaves the edited plants with marker genes termed 'foreign DNA'. In sexually propagated crops, selfing or outcrossing can easily segregate these foreign DNA markers from transformed plants but is more difficult in vegetatively propagated crops like grapevine. This makes it more challenging to embrace the conventional transgenic approach for transgene-free gene editing in grapevine. A recent technique is to edit the genes through the delivery of Cas9 and single guide RNA as ribonucleoproteins (RNP) into regenerable tissues. As proof of concept, we proposed to edit MLO susceptibility powdery mildew genes through a combination of plasmid and RNPs in the microvine to produce transgene-free mildew-resistant plants. Loss-of-function mutations in 'Mildew resistance Locus O' (MLO) genes confer durable broad-spectrum resistance against Powdery Mildew. We have tested Cell-Penetrating Peptides (CPP) to facilitate the entry of the RNP into somatic embryogenic calli. VitviMLOs 3, 4, 13, and 17, the closest orthologs of MLO genes associated with powdery mildew resistance in other species, were targeted for the editing using plasmid. We are currently propagating ~200 transformed, potential *mlo*-mutant clones of microvine in preparation for disease resistance tests on the regenerated seedlings. Preliminary genotyping results in CRISPR-induced indels/deletions/substitutions in 90% of clones examined. Embryogenic calli will be produced from the gene-edited powdery mildew-resistant mutants and the transgene cassette will be excised from the genome by CRISPR RNP delivery as part of the second phase of the project. Experiments conducted to assess the efficacy of CPPs in RNP delivery into microvine embryogenic cells, in preparation for the second transgene elimination phase, confirmed the editing of the target gene.

Developing Transgene-Free Genome Editing Tool for Clonally Propagated Crops Through Homology-Directed Repair Pathway

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Gene editing technologies that couple the transgene-free approaches and improved Homology-Directed Repair (HDR) pathway for precision editing comply with the recent USDA regulations to be classified as non-GMO and ensure the successful implementation and commercialization of the improved varieties. Recent developments enable researchers to avoid foreign DNA insertions by delivering the CRISPR components as a ribonucleoprotein complex (RNP). In addition, Cas9 fusions to accommodate DNA repair templates enhance HDR frequency. RNP-mediated gene editing has been successfully used in many plant species, including grapevine, through the protoplast transfection and regeneration of edited protoplasts, which in most clonally propagated crops such as grape, citrus, and apple, is extremely difficult. So, to perform gene editing through CRISPR RNPs, we planned to use Cell-Penetrating Peptides (CPP), which were shown to deliver protein cargos into intact plant cells efficiently. We had shown that CPPs could be fused to Cas9 protein through a bioconjugation or noncovalent complexing and delivered into microvine embryogenic callus. To improve HDR-mediated gene editing, we use Cas9 fused with Agrobacterium VirD2 protein, which can bind to DNA strand through a short recognition sequence, to tether the donor template. The proximity of the donor template with desired changes to the double-stranded break site is an essential factor in improving the HDR frequency. As a proof of concept, we plan to make a precise single base substitution (195T>C) in the eGFP gene that will shift its fluorescence features towards the blue spectrum (BFP) using the embryogenic callus of the microvine expressing GFP. For economically important fruit crops such as grapevine, which are clonally propagated and have long generation cycles, there is a critical need to develop techniques to deliver CRISPR RNPs with enhanced precision-editing into regenerable tissues to advance the use of gene-editing technology.

Characterization of Red Blotch Virus (GRBV) Early Infection Dynamics for the Identification of RNAi-targeted Hotspots

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Red Blotch Virus (GRBV) is a single-stranded DNA geminivirus that has been identified as the etiologic agent of the Grapevine Red Blotch disease. During early phases of infection, viral-derived small interfering RNAs (vsiRNAs) are generated via the plant RNA interference (RNAi) machinery, which represents one of the first antiviral responses activated by the plant. This results in either the viral mRNA degradation, known as Post-Transcriptional Gene Silencing (PTGS), or in the viral DNA methylation, known as Transcriptional Gene Silencing (TGS). To characterize these early responses, we have designed a vacuum-assisted agro-infiltration protocol for inducing the systemic infection in tissue-cultured plants. These were collected every three days for total RNA extraction. Real-Time PCR (RT-PCR) assays on samples from 0 to 18 days post-inoculation (dpi) revealed a significant peak in viral replication as early as 3-12 dpi. We used these samples for also targeting Dicer-like 2 (DCL2) encoding gene, which is directly involved in the PTGS response, for validating the activation of the plant RNAi machinery. Subsequently, DCL2 expression dynamics confirmed that the plant defense machinery has been activated during the viral replication peak at 3-12 dpi. That being said, the next steps will be focused on small RNA and bisulfite sequencing of the samples from plants collected between 3 to 12 dpi, to identify respectively the vsiRNAs, and the viral genomic regions subject to methylation or TGS. These two layers of information will contribute to the characterization of viral "**hotspots**" targeted by the plant RNAi machinery. On these, relies the further designing double-stranded RNA sequences, that will be use in the developing a new generation of RNAi-based biopesticide for GRBV. Such system could represent a long-term, reliable and economically sustainable alternative to intense labor management and/or removal of infected plants, that are currently the only options available for viral infection management.

Application of Smoke Containing ^{13}C Isotopes to Wine Grapes for Chemical Characterization of Smoke Influences in Wine

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Smoke exposure to wine grapes has been a growing concern for vintners, winemakers, and consumers as many vineyards the United States and Australia reside in areas where wildfire intensity has been increasing since at least the 1950s. Wine has shown to be particularly sensitive to smoke exposure, often acquiring an "ashy", "burnt rubber", or medicinal flavors or aromas after smoke exposure. To better serve the industry, we have designed a method to perform a more thorough inventory of chemical compounds associated with smoke. A fuel source for smoke, barley was chemically labeled using the stable isotope of $^{13}CO_2$. Upon assimilation after 10 days of $^{13}CO_2$ exposure, the ^{13}C is expected to be incorporated into the smoke precursor compounds, such as lignin. After drying, the barley was analyzed for ^{13}C content using IRMS, burned, and the smoke piped "cold" to chardonnay and pinot noir grapes, grown in Willamette Valley, Oregon, post-harvest in a sealed container designed for this study. After the $^{13}CO_2$ exposure period, IRMS data revealed incorporation of ^{13}C of 4.64±1.73% compared to ^{12}C content over the 1.08±0.01% ^{13}C in the untreated barley. Partition studies show the newest growth during treatment achieved as high as 7.47±2.60% and 6.65±2.26% in the leaves and stems, respectively. Chemical analysis using RP-HPLC, mass spectrometry, and ^{13}C-NMR will be used to elucidate the chemicals potentially responsible for smoke flavors and aromas in affected wines. Herein, we show the methodology for producing and applying smoke containing isotopically labeled compounds.

Understanding of Retronasal Attributes Associated with Smoke Taint in Wildfire Affected Wines and Determination of Proper Inter-Stimulus Protocol for Sensory Analysis

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As wildfire occurrence has increased around the world, the smoke produced from them has begun to pose unique issues to wine industry. The volatile organic compounds carried within smoke when absorbed by grapes impart unpleasant smokey and burnt flavor, with a lingering ashy finish. During sensory analysis, residual sensations can lead to carryover bias, which can cause increased perceived intensity ratings when evaluating many samples in sequence. Since there is an observed lasting nature with these smoke related flavors, carryover must be mitigated to ensure accurate identification and sensory analysis of smoke affected wines. Previous work indicated that a 1 g/L pectin rinse solution is effective in combatting this bias but requires a lengthy 2-minute separation between samples. The purpose of this work was to evaluate other rinsing strategies to determine if there was an option that was equally as effective, but more efficient. Using a fixed-time point evaluation system, the progression of the intensity of smoke related attributes (smokey, ashy) and typical wine attributes (mixed berry, floral) with various inter-stimulus rinsing protocols (pectin, ethanol, lipids, dextrose) were evaluated. Of these rinses, the 4 g/L dextrose solution was the most efficient in clearing smoke flavor perception, requiring 90 s to return the mouth to baseline conditions. Additionally, this work identified retronasal flavor standards that are representative of the flavors found in smoke-affected wine that can be used to better understand the in-mouth sensations of the alterations introduced by smoke. Overall, this study provided greater insights into the sensorial impact of wines produced from wildfire affected grapes. The conclusions can be used to guide effective sensory practices in future analysis and understanding of wildfire affected wines.

Funding: USDA-Agricultural Research Service (ARS) project number 2072-21000-057-00D

Evaluation of Novel Coatings to Prevent Smoke Phenol Absorption in Pinot noir Grapes

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Wildfires have been a continuing threat to the wine industry over the years. Smoke exposed grapes result in smoky, ashy, and medicinal sensory characters that are undesirable in wine. To combat this problem, new mitigation techniques are needed to preserve the quality of the grapes. In response to the lack of successful smoke taint reduction techniques, innovative coatings were developed to prevent smoke compounds from entering the grapes. Pinot noir grapes were grown in Woodhall III Vineyards in Oregon and harvested at maturity. After harvest the grapes were coated with four different coating treatments by spraying. Cellulose nanofiber (CNF) was used as a coating forming matrix, and other functional ingredients were incorporated into CNF to develop four different types of films. Uncoated grapes were used as a control. ^{13}C labeled barley was used as a fuel source to create the smoke in specially designed cages covered in low density polyethylene greenhouse film. The grapes were smoked for 6 hours, achieving a constant smoke density between 20 to 100 mg/m^3 for smoke particles >1 μm. After the grapes were smoked, half were washed to determine if the smoke compounds bind or are blocked by the coatings. GCMS was used to determine the amount of smoke compounds in the grapes. The results of this study will determine if the novel film coating is a prospective preventative measure to stop wildfire smoke from entering the grapes. Therefore, when a wildfire occurs the wine industry can prevent smoke taint and the potential loss of product.

Funding: Specialty Crop Research Initiative [grant no. 2021-51181-35862/project accession no. 1027470] from the USDA National Institute of Food and Agriculture

Impact of Grapevine Red Blotch Disease and Irrigation Treatments on Pinot noir Grape Grape Development and Wine Quality

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Grapevine red blotch disease (GRBD) is caused by grapevine red blotch virus (GRBV). GRBD delays grape berry maturity and sugar accumulation and may have great effects on fruit and final wine composition. To better understand the impact of GRBD on grape and wine quality, a three-year field study was conducted in Rogue Valley AVA, Oregon through 2018 to 2020. Two irrigation treatments (well-watered (W) and water deficit (D)) and two vine statuses (GRBV-infected (RB+) and non-infected (RB-)) were performed in a randomized complete block design. Grapes were collected during berry ripening till harvest (one week after harvest in 2019 and 2020) from RB+ and RB- grapevines. Wines were made in triplicates from 2018-2020 grapes under treatments D+ (D and RB+), D- (D and RB-), W+ (W and RB+), and W- (W and RB-) according to the standard protocol of OSU winery. Berry total soluble solids, berry organic acids, berry phenolics, berry free and bound form C_{13}-norisoprenoids, wine phenolics, and wine flavor profiles were investigated. RB+ grapes had a lower level of total soluble solids and malvidin-3-glucoside during berry development. Higher citric acid level was observed at harvest in RB+ grapes compared to RB- grapes in all three years. No consistent results were found on grape derived aroma compounds among years. W+ wines showed significant lower monomeric anthocyanin and total phenolic content compared to W- wines in 2018 and 2019 and no statistically differences were observed between D+ and D-. Volatile compounds can be affected by both the health status and irrigation treatments of grapevines. W treatment may enhance the levels of some volatile compounds (such as β-damascenone, β-ionone, linalool, and nerol) in RB+ wines compared to D treatment. While the patterns were not consistent among three years, which suggested vintage was also an important factor on wine volatile profile.

Funding: California Department of Food and Agriculture and American Vineyard Foundation

Revealing the Sensory Impact on Different Levels of Esters and Volatile Thiols in White Wines

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Tropical fruit aromas, such as passionfruit, guava, and grapefruit are present in a wide array of white wines and studies revealed that consumers enjoy these aromas. Substantial work has revealed that these aromas are imparted by volatile thiols. More recent evidence, however, suggests that tropical fruit aromas are also caused by other aroma compounds besides thiols, such as fermentation esters or the interactive effect between these volatile families. Therefore, the objective of this study was to investigate the effects of esters and thiols on the fruitiness aroma perception of white wines. Pinot gris wine was produced at the OSU research winery and was dearomatized using Lichrolut® EN. Combinations of fermentation volatile compounds were added to the wine, forming the aroma base. Treatment wines were composed of additions of four levels of thiols (none, low, medium, and high) and three levels of esters (none, low, medium) in a full factorial design. Samples were subjected to sensory analysis where forty-nine white wine consumers responded to Check-All-That-Apply (CATA) to find aroma descriptors that most differentiated the wines. Following the results obtained by CATA, samples were subjected to a Sensory Descriptive Analysis (SDA) panel where thirteen trained panelists evaluated the intensity of eight aroma attributes. Thiol treatments without the presence of esters contributed to earthy and grassy aromas. Overall, tropical fruit aromas were detected in the several treatments containing esters and esters + thiols. Differences in the quality of tropical fruit aroma were observed across the distinct levels of esters and thiols in the samples. This study showed that both aroma families are important for tropical fruit aroma perception in white wines. Therefore, grape growers and winemakers should adapt viticultural and winemaking conditions to increase the concentrations of these two aroma families to enhance this aroma characteristic when desired.

Funding: American Vineyard Foundation

Smoke Compounds Evolution During Again

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Smoke exposure in wine is an increasingly important topic to research, affects all stages of operations, from winegrowing, winemaking and marketing. Smoke can cause a terrible ashy flavor in the wine. Smoke taint is caused by a various volatile free phenol that are present in smoke, with the most common indicators being guaiacol, 4-methylguaiacol, o-cresol, p-cresol, and m-cresol. These phenols bind to sugars within the grapes, and then are released upon fermentation, causing the smokey "off flavor". They can be detected by gas chromatography mass spectrometry (GC-MS). While a fair amount of research has been done to mitigate the issue, the negative sensory impacts seem to regularly return on aging of the wine. This research focuses on how smoke compounds change and evolve during this aging process. 82 wine samples from the 2020 wildfire season were analyzed twice about a year apart, using GC-MS, with a solid phase micro extraction technique (SPME). 10ml samples and 10ul of internal standards were injected into the GC-MS, and concentrations of each free volatile compound were calculated using ChemStation software. Overall, the results showed very minimal overall change, with the majority of concentrations decreasing slightly after a year of aging. 4-methylguiaciol concentration did increase over the year, but not a significant amount.

Effect of Fermentation Temperature Gradient and Skin Contact on Ester and Thiol Production and Tropical Fruit Perception in Chardonnay Wines

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Wines with tropical fruit aromas have become increasingly more available, making it more important to understand the compounds that cause these tropical fruit aromas in wine. Previous work using micro fermentations showed higher fermentation temperatures and increased time on skins resulted in an increase in thiol and ester compounds post fermentation. The aim of this work was to scale up those micro fermentations that successfully increased thiols and esters. Descriptive sensory analysis was conducted to determine the aroma profile associated with the thiol and ester content of the wines. Future work will evaluate the consumer acceptance of these wines, as very little information is available linking tropical fruit aroma on consumer preference. The four treatments tested were 1) Control fermentation at 13°C with no skin contact; 2) fermentation at 13°C with 18 hours of skin contact; 3) fermentation temperature gradient by time (20°C for 4 days then reduced to 13°C) with no skin contact; and 4) fermentation temperature gradient by time with 18 hours of skin contact. A change in winemaking scale did not alter the pH, residual sugar, or alcohol of the wines. Post filtration, thiol, ester, and thiol precursor analysis will be conducted. Sensory analysis used check-all-that-apply (CATA) to determine the best attributes to focus on for descriptive analysis, followed by Just-About-Right (JAR) testing to determine the best training standards for the terms determined from CATA. Descriptive analysis using trained panels was conducted to determine the intensity of the different aromas presented in the wines. Overall, this study will display if specific winemaking processes can significantly influence the tropical aroma of Chardonnay through increased levels of thiols and esters. Understanding the causes of tropical fruit aromas in wine and processes that alters these compounds is necessary to ensure winemakers can achieved tropical fruit quality consistently.

Funding: Northwest Center for Small Fruit Research

Using non-*Saccharomyces* Yeast to Reduce Spoilage by Acetic Acid Bacteria During Cold Soaking

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This study investigated an alternative technique to prevent spoilage during cold soak through the addition of a high population of a non-*Saccharomyces* yeast as a bio-protectant. The use of non-*Saccharomyces* yeast had previously been demonstrated to reduce growth and production of spoilage products by *Hanseniaspora uvarum* so this work focused on whether similar results would be seen for the spoilage bacteria *Acetobacter pasteurianus.* Two commercially available non-*Saccharomyces* yeast cultures, *Metschnikowia fructicola* Gaiia™ and *Torolaspora delbrueckii* Prelude™ were assessed for their ability to suppress the growth and acetic acid production of *A. pasteurianus* during a simulated cold soak in a model grape juice. Initial experiments at 8°C resulted in very little growth of *A. pasteurianus* indicating that *A. pasteurianus* did not grow well at this temperature. Because of this finding, additional experiments were conducted at 10°C and/or 13°C. At warmer temperatures, higher growth of *A. pasteurianus* was observed but only small amounts of acetic acid were produced when *A. pasteurianus* was grown by itself. In contrast, higher acetic acid production was observed when *A. pasteurianus* was grown in co-culture with either *M. fructicola* Gaiia™ or *T. delbrueckii* Prelude™. For example, at 13°C *A. pasteurianus* only produced 78 mg/L of acetic acid, while in co-culture with *T. delbrueckii* Prelude™ 300 mg/L acetic acid was produced. The cause of this increase in acetic acid production was investigated by measuring ethanol production during the cold soak. While *A. pasteurianus* did not produce any ethanol, both non-*Saccharomyces* yeast produced greater than 3.5 g/L ethanol during a six day cold soak at 13°C. Because *A. pasteurianus* produces acetic acid from ethanol the production of ethanol by *M. fructicola* Gaiia™ or *T. delbrueckii* Prelude™ led to increased acetic acid production when *A. pasteurianus* was grown in co-culture with these yeast. Overall, colder cold soak temperatures were sufficient to repress *A. pasteurianus* growth while adding a non-*Saccharomyces* yeast as a bio-protectant at higher cold soak temperatures was counterproductive as ethanol production by these yeast led to higher acetic acid production by *A. pasteurianus*.

Funding: Oregon Wine Research Institute and Northwest Center for Small Fruits Research

Interactions Between *Oenococcus oeni* and *Brettanomyces bruxellensis* During Malolactic Fermentation

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A rapid malolactic fermentation (MLF) initiated by inoculation of *Oenococcus oeni* is a useful strategy to prevent *Brettanomyces bruxellensis* spoilage by minimizing the length of time wine is not protected by SO_2. This project investigated an additional benefit of conducting a rapid MLF, the prevention of *B. bruxellensis* growth due to inhibitory interactions with *O. oeni*. The ability of commercial *O. oeni* strains to inhibit *B. bruxellensis* growth at the end of MLF in Pinot noir wine was tested. All ten *O. oeni* strains tested inhibited *B. bruxellensis* UCD2049 growth and volatile phenol production with some *O. oeni* strain variation observed. The potential mechanism of this inhibition was investigated by using a dialysis membrane to physically separate *O. oeni* and *B. bruxellensis* cells in wine but allow free movement of nutrients and other potential inhibitory compounds. Physical separation of *O. oeni* cells from *B. bruxellensis* cells relieved the inhibition of *B. bruxellensis* by *O. oeni* that occurred when the two microorganisms were present together suggesting that inhibition was not due to nutrient depletion by *O. oeni* or an inhibitory compound. Instead, these results provide evidence that the inhibition of *B. bruxellensis* by *O. oeni* was due to cell-cell contact. The effect of MLF timing on *B. bruxellensis* inhibition was then determined. While *B. bruxellensis* was strongly inhibited if inoculated at the end of MLF, no inhibition of *B. bruxellensis* occurred when inoculated at the beginning of MLF. The sensitivity of additional *B. bruxellensis* strains to *O. oeni* was also determined. While *B. bruxellensis* UCD2049 populations declined rapidly when inoculated into Pinot noir wine that had just completed MLF with *O. oeni* Alpha, growth of seven other *B. bruxellensis* strains tested was not impacted. Subsequent experiments showed that ethanol tolerance differences between *B. bruxellensis* strains may have played a role in their sensitivity to repression by *O. oeni*.

Funding: Oregon Wine Board

Free and Bound Volatile Phenols in Smoke-Exposed Wines- Biomarkers, Machine-Learning, and Model Prediction

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Smoke-derived taint has become a significant concern for the U.S. wine industry. It is an off-aroma describing the wine with smoky, medicinal, and ashy characters. This unpleasant aroma is caused by grapes or grapevines exposed to bushfire smoke before grape harvest. During smoke exposure, volatile phenols (VPs) can be absorbed by grapevine and the grapevine can convert these volatile phenols (VPs) to VP-glycosides. VPs such as guaiacol, 4-methylguaiacol, m-, o-, and p-cresol and the corresponding VP-glycosides could have an adverse effect on the quality of the wines. Free and total VPs, as well as VP glycosides in smoke-exposed wines were investigated by GC-MS and LC-high-resolution accurate tandem mass spectrometry (HPLC–HRMS/MS) and compared with control wines without smoke exposure. GC-MS analysis showed that the concentration of guaiacol, 4-methylguaiacol, m-, o-, and p-cresol in smoke-exposed wines varied widely but were generally higher than those in control wines. Total p-cresol (free + bound form) presented the most significant difference between control and smoke-exposed wines and was the most discriminant compound for smoke exposure, followed by free form o-, p-, and m-cresol. LC-MS/MS analysis tentatively identified thirty-five VP-glycosides at higher levels in smoke-exposed wine.

Furthermore, fifteen VP-glycosides were significantly higher in smoke-exposed wines ($p < 0.05$) and provided total discrimination among groups. A hexose-pentose-4-methylguaiacol presented the most significant difference (FC=4.60, p=2.37×10-8). Machine-learning and model prediction were used to evaluate volatile phenols as biomarkers for wine exposure. In addition, volatile phenolic compound evolution during aging was investigated. The results showed significant increases during the first three months and only increased slightly afterward. Comprehensive two-dimensional 2D GC X GC-time of flight-MS and GC/MS-olfactometry analysis were also used to identify additional smoke compounds in the wine. The results were inconclusive.

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Isolation and Characterization of Polysaccharides from Pinot noir Grape and Wine Yeast *(Saccharomyces cerevisiae)*

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The organoleptic properties is recognized as an important factor in wine quality. The polysaccharides from Pinot noir grape and *Saccharomyces cerevisiae* were the main polysaccharides component and also play an important role in wine characteristics. Further studies on the characterization of polysaccharides and their interaction with wine volatile compounds are necessary to understand the organoleptic properties. In this study, the polysaccharides fractions from Pinot noir grape and *Saccharomyces cerevisiae* were collected by loaded onto a DEAE Sephacel column (5.5 cm × 60 cm) and then eluted with 1.2 L of aqueous NaCl solution (0 M, 0.1 M, 0.3 M, 0.5 M) at a flow rate of 5 mL/min. Subsequently, the different fractions of polysaccharides were obtained by isolating with the phenol-sulfuric acid method and concentrating by ultrafiltration with a molecular weight cut-off of 2 kDa. The characterization of polysaccharides was determined by ultraviolet-visible spectroscopy with a scanning range of 190-600 nm and attenuated total reflection-Fourier transform infrared spectroscopy in the wavelength range of 400-4000 cm^{-1}. It showed different structural features of polysaccharides in ATR-FIIR spectroscopy. The level of absorption peaks at 260 nm and 280 nm (nucleic acid and protein characteristic peak) in purified polysaccharides were lower than crude polysaccharides in UV-vis spectrum. FT-IR spectra proved the structural features of the purified polysaccharides.

Keywords: Polysaccharides, Isolation, Characterization, Interaction

Determining the Chemical Components that Cause Sensory Qualities in Wine

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Wine is a complex beverage, and the vast majority of quality aspects are related to multiple components within the system. Determining relationships between flavor chemistry and sensory perception in complex mixtures, such as wine, is very challenging. Regression based methods used to relate chemical composition to sensory perception do not take into account the many possible ways in which volatile compounds may contribute to aroma. Work in the Tomasino lab has focused on how different combinations and concentrations of compounds influence various qualities in wines. Details will be provided on the causes of tropical fruit aroma in white wines, how different combinations of monoterpenes change the aroma of Viognier and Pinot gris wines, the chemical combinations that cause red berry fruit and dark berry fruit aromas in Pinot noir wine and the dominance of phenolic compounds in the perception of Pinot noir wine mouthfeel.

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Glove Fits Really Well! New Methods in Monitoring Grape Powdery Mildew

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Detecting grape powdery mildew (GPM) in the field before it becomes an issue can take a trained scout hours of searching while still having a small chance of detecting disease early in the epidemic. Impaction spore traps have been used for disease monitoring but can be expensive and inflexible to operate with an unknown (but limited) sampling range. Workers in the vineyard manipulating the canopy potentially can have GPM spores deposited onto their gloves which can be collected with a cotton swab (glove swab) for qPCR detection. The glove swabs were compared to both visual assessment collected 'leaf swabs' (n= 920) and spore traps (n= 206) across three growing seasons. The results show that glove swabs are far more sensitive than a leaf swab (e.g., 97% vs 51% sensitivity in 2019) while providing very similar disease information to a spore trap (e.g., 90% vs 96% sensitivity in 2019). In addition to their use for monitoring GPM, these methods can be used to monitor for FRAC group 11 (aka QoI or Strobilurin) fungicide resistance. In the Oregon fields tested, there was a FRAC 11 resistance incidence between 45-62%. Additionally, even in fields where no FRAC 11 products were used, the proportion of resistant samples increased within a season. In 5 fields where FRAC 11 products were used next to fields with a different FRAC group applied (though they were otherwise managed the same), each field was able to achieve a similar level of control, despite pre-existing low levels of FRAC 11 resistant GPM present. With disease and resistance monitoring, FRAC 11 products may still be useful tools for GPM management and this research provides additional monitoring techniques to make monitoring easier for more vineyards.

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Developing a Spray Induced Gene Silencing Method for the Control of Grape Powdery Mildew *(Erysiphe necator)*

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Spray Induced Gene Silencing (SIGS) is a new technique for gene silencing without genetic modification by the exogenous application of double-stranded RNA (dsRNA). The downregulation of specific plant susceptibility genes can lead to broad-spectrum resistance to pests. Our project aims to develop a methodology to turn off a group of grapevine susceptibility genes (Mildew Locus O) to induce resistance to the fungal pathogen *Erysiphe necator* (Powdery Mildew). As a first step, we examined whether the amplified cDNA regions of the MLO genes generated a reduction in their expression *in planta*. We conducted a root soaking experiment twice with 8-week-old microvine plants growing in a liquid medium containing a mixture of dsRNA at 20ng/μL, targeting five MLO candidate genes. The effects of RNA interference were analyzed using real-time PCR assays. We did not observe any change in the expression of the native MLO genes for four of our five candidates after 3-, 7-, and 14-days post-incubation. Instead, *VitviMLO17* showed an increased and reproducible accumulation after incubation with dsRNA expected to reduce its expression. The second experiment evaluated the ability for systemic movement of fluorolabeled dsRNA molecules in root and leaf petioles of same-age grapevine plants. The initial imaging was accomplished from root tissues of tissue-cultured plants after seven days of incubation with a liquid growing medium containing the labeled dsRNA. The analysis by confocal microscopy revealed the presence of dsRNA in root hairs and central root cells. Additional assays will be needed to further confirm the uptake and the movement of the molecules towards the upper part of the plants. These results indicate that further assays are required to a) verify dsRNA sequences are capable of silencing target genes via RNA-interference and b) validate cellular uptake of dsRNA species by root cells via root soaking.

Funding: Northwest Center for Small Fruit Research

Potential Insect Vectors of Red Blotch Virus in Southern Oregon

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Insects suspected of being vectors of red blotch are collected in Southern Oregon vineyards and undergo PCR testing for the presence of the red blotch virus. The species that have most often tested positive for red blotch are cixiids; the leafhopper, *Colladonus* sp.; and the treehopper, *Stictocephala bisonia*. However, an insect testing positive for red blotch only indicates that it can pick up the virus and not that it can transmit it. Transmission testing on live plants is a lengthy and often frustrating process. An in vitro technique was evaluated in the fall of 2021. Insects are placed in a tube with a sucrose solution under parafilm and allowed to feed on the solution through the parafilm. Then the solution is tested with PCR for the virus. A positive test indicates that the insect transmitted the virus via feeding into the solution. One out of two *S. bisonia* collected along the edge of a red blotch infested vineyard tested positive for the virus as did the solution that the positive insect fed on.

The effect of clean cultivation on treehopper feeding activity was examined in two replicated trials. The first was conducted in a grower vineyard in 2019 and compared cultivation with standard mowing practice. The second trial was established in 2020 at SOREC's Lombard research vineyard and compared cultivation to a planted cover crop mix of grass and birdsfoot trefoil. The results of both trials showed no statistically significant difference between the treatments. However, treehopper feeding activity was consistently lower in the cultivated plots. When treehopper feeding was measured in the research vineyard plot in 2021, the number of girdles per vine was 0.17 in the cover crop treatment versus 0.05 in the cultivated treatment, a reduction of 70%.

Funding: Red Blotch SCRI Grant

Invasive Mealybug Distribution Survey in Oregon: Gill's and Vine Mealybugs are Here!

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Gill's mealybug (Gill's MB, *Ferrisia gilli*) is an emerging pest of multiple crops. In Oregon, the crop most at risk for damage is grape. One California study of Gill's MB impacts documented infestation of up to 42% of grape clusters. Gill's MB has been in California since around 1968, but it was first detected in Oregon in 2014, in Jackson County. It was most likely brought to a vineyard on infested stock for planting from California. Since then, Gill's MB has persisted and spread. Gill's MB could pose a regulatory problem for growers shipping to uninfested states or countries. Gill's MB is also a vector of grapevine leafroll viruses, a significant concern for growers. If no action is taken, this pest will spread through suitable growing areas throughout the state. In addition to grape production, it may impact both the nursery and fruit industries. ODA's Insect Pest Prevention and Management program has begun a project to delimit the area infested by Gill's MB with the intent of educating Oregon grape growers about this pest and management practices to limit its spread. Delimitation will be conducted by ODA staff in Jackson County and, with the assistance of vineyards, throughout the rest of the state. No lure is available for this pest, so visual survey is the only option. In the process of preparing for this project, ODA detected vine mealybug (VMB, *Planococcus ficus*), a quarantine pest and one of the primary pests of grape production in California. VMB is an excellent vector of grape leafroll disease. ODA will attempt to determine the extent of the infestation of VMB while conducting the Gill's MB survey. The work is being implemented in cooperation with OSU extension.

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Efficacy of Sulfur and Biological Fungicide Regimes on Management of Grape Powdery Mildew

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During the 2021 growing season micronized sulfur and biological fungicides were applied during critical periods for grape powdery mildew (GPM) management on Pinot noir and Pinot gris vines at the Botany and Plant Pathology Field Laboratory in Corvallis, OR. In addition, tank mixes of biological fungicides and low rates of sulfur were used for GPM management on Chardonnay vines. In previous studies, use of biological products all season generally resulted in poor powdery mildew control. The goal was to evaluate the efficacy of biological fungicides and achieve practical levels of GPM control. In Pinot noir and Pinot gris trials, biological fungicides were evaluated by application only during the critical period of flowering (Pinot gris), or before and after flowering (Pinot noir) with 5lb/Acre (5.6kg/ha) sulfur applied outside of those timeframes. In a Chardonnay trial treatments focused on evaluating whether tank mixes of biological fungicides with 2.5lb/Acre (2.8 kg/ha) sulfur would provide better control than using the low rate of sulfur alone. All fungicide treatments within each individual trial had similar GPM leaf incidence as measured by area under disease progress curve (AUDPC). Fungicide regimes in Pinot noir and Pinot gris trials resulted in 6-10% and 13-19% cluster severity, respectively, with non-treated controls resulting in 75% severity. In the Chardonnay trial, average cluster severity ranged from 8%-20% compared with 27% for the 2.5lb/A (2.8 kg/ha) sulfur control. These data suggest that applying biological fungicides before GPM is established in a vineyard early in the season and using known efficacious fungicides at bloom, in this case sulfur, was the most effective strategy at managing GPM cluster infections implemented in the trials. Further examination of this strategy with other products as well as other tank mix regimes will be investigated in 2022.

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UV-C Light for Grapevine Disease Management

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Germicidal ultraviolet spectrum C (UV-C) light is a promising integrative management tool for grapevine powdery mildew that could help reduce the quantity of fungicides applied to manage the disease. Our objective was to determine the effects of nighttime UV-C application on grapevine powdery mildew pathogen, *Erysiphe necator*. In lab studies, we suspended two, 36-Watt low pressure discharge UV-C lamps above an adjustable conveyor system to apply controlled doses of UV-C to *E. necator* conidia on Gelzan media filled 24-well plates. The half-maximal UV-C dose to inhibit conidia germination varied across isolates from 98 to 245 joules per square meter (J/m^2) which suggests that there may be cellular mechanisms that influence the tolerance of powdery mildew to UV-C. In vineyard studies, we deployed a tractor-drawn, over-canopy array of 24, 55-Watt UV-C lamps to expose VSP trained Pinot Noir vines to weekly nighttime (1 hr. after sunset) UV-C applications of 80 or 120 J/m^2 in 2020 and twice weekly nighttime UV-C applications of 120 or 200 J/m^2 in 2021. UV-C applications were performed in conjunction with different fungicide programs. Our results show that once and twice weekly UV-C applications both led to a significant reduction in the overall powdery mildew leaf incidence epidemic. Twice weekly UV-C application also reduced cluster incidence and the amount of detected inoculum from glove swab samples. The Brix, pH, anthocyanin, and phenolic content of whole berry homogenate from UV-C treated grapes was not significantly different from untreated grapes. These results suggest that UV-C treatments, in conjunction with fungicide programs and other integrated pest management strategies, may have the potential to improve disease management of grape powdery mildew, but still needs to be made more economically feasible; potentially by using autonomous robotic platforms.

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Thank you for being a part of the Oregon Wine Research Institute Grape Day!

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