

Oregon Wine Research Institute

Viticulture & Enology

Technical Newsletter

Summer 2018



Welcome to the Summer 2018 Newsletter

Our latest edition of the OWRI Technical Newsletter contains research updates and a comprehensive list of publications summarizing research conducted by faculty of the Oregon Wine Research Institute at Oregon State University. Dr. R. Paul Schreiner, USDA-ARS Research Plant Physiologist, opens the newsletter with a research update on Pinot noir nutrient needs and tissue test guidelines for nitrogen, phosphorus, and potassium. Dr. James Osborne, OSU Enology Extension Specialist and Associate Professor, along with Dr. Michael Qian, OSU Professor, and Dr. Elizabeth Tomasino, OSU Associate Professor, provide valuable information on their research of the role of grape microflora on Pinot noir aroma. Lastly, Drs. Alexander Levin and Achala KC, OSU-Southern Oregon Research and Extension Center (SOREC) Assistant Professors, provide a timely article about deficit irrigation and grapevine red blotch virus concerns.

To read this newsletter online, visit the OWRI website at <https://owri.oregonstate.edu/oregon-wine-research-institute/extension-resources/owri-newsletters> and bookmark this page for future reference.

Cheers,
The OWRI Team

Pinot noir Nutrient Needs and Tissue Test Guidelines for Nitrogen, Phosphorus, and Potassium

Dr. R. Paul Schreiner, Research Plant Physiologist, USDA-ARS

Grape growers are encouraged to monitor vine nutritional health by using tissue tests performed on leaf blades or petioles and comparing these to nutrient level guidelines. However, the guidelines used by testing laboratories have not been calibrated for Pinot noir production in Oregon, and there is still debate on which tissue to use to best monitor vine nutrient status. This article provides a summary of research findings from my lab on the nitrogen (N), phosphorus (P), and potassium (K) requirements to produce premium Pinot noir wines in western Oregon. New tissue guidelines for N, P, and K are proposed based on these studies where nutrients were carefully controlled and fruit yield was maintained between 2.2 to 3.5 tons per acre. In addition, results from this work provide guidance on tissue selection for monitoring vine nutrient status.

IN THIS ISSUE

- * Pinot noir Nutrient Needs and Tissue Test Guidelines for Nitrogen, Phosphorus, and Potassium
- * Unravelling the Mysteries of Cold Soaking: The Role of Grape Microflora
- * How do deficit irrigation and grapevine red blotch virus influence disease severity, water stress, yield, and fruit composition?
- * Research Publications

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Summary of Oregon NPK Trials

Nitrogen. Nitrogen supply had the greatest overall impact on Pinot noir productivity and berry and wine chemistry compared to P and K in two NPK trials conducted in Oregon. Reducing N supply had a nearly immediate impact on vine canopy size (leaf area and pruning weights) in both experiments (Schreiner et al. 2013, 2018). Nitrogen availability is the driver of vegetative growth, as long as ample soil water is also available. Nitrogen status had much less impact on fruit yield, meaning that N supply can be used to control vigorous canopies and reduce canopy management costs without reducing yield when vines are cropped at a similar yield as in this research. Lowering N availability will, however, result in lower yeast assimilable nitrogen (YAN) concentrations in the fruit or must. Must YAN levels were slightly more sensitive to vine N status than vegetative growth. Therefore, lower YAN will result if you use a lower N supply to reduce canopy size. This problem may not be as significant as previous research has suggested. The minimum YAN requirements for yeast to complete fermentation for wine production is reported to be from 140 mg to 275 mg N/L for a 24 Brix must (see Schreiner et al. 2018 and references therein). A minimum YAN of 140 mg N/L is commonly used in the industry. However, fermentations of Pinot noir were not slower in our studies until YAN was about 100 mg N/L, and the lowest YAN musts (25 mg N/L) fermented to dryness (Schreiner et al. 2018). Stockert et al. (2013) also noted that Merlot musts completed fermentation with YAN levels as low as 60 mg N/L. These findings indicate that YAN values for red cultivars do not need to be as high as 140 mg N/L, and we suggested that 100 mg N/L is a better target for Pinot noir (Schreiner et al. 2018). As a result we based tissue N guidelines for Pinot noir on obtaining must YAN of 100 mg N/L and where yield was not reduced compared to the high N control vines (Table 1). The critical leaf blade N at veraison that achieved this was 1.90% N, but to account for potential sampling and laboratory error, a safe level of 2.00% N is proposed (Table 1). It should be noted that when fruit had YAN levels of 100 mg N/L, canopy growth was reduced by about 30% compared to the control vines.

Critical values for leaf blade N at bloom, and for petiole N at bloom and veraison, are provided also.

Table 1. Nitrogen, Phosphorus and Potassium Tissue test Guidelines for Pinot noir derived from vines grown in a pot-in-pot system on a VSP trellis and carrying between 2-4 tons per acre.

Nutrient	Time / Tissue	Concentration (% of dry weight)		
		Critical level	Safe/Healthy	Excess
Nitrogen	Bloom / Leaf blade	2.20	> 2.40	4.20
	Bloom / Petiole	0.55-0.70*		
	Veraison / Leaf blade	1.80	> 2.00	2.50
	Veraison / Petiole	0.35-0.45*		
Phosphorus	Bloom / Leaf blade	0.17	> 0.18	
	Bloom / Petiole	0.12	> 0.14	
	Veraison / Leaf blade	0.10	> 0.11	
	Veraison / Petiole	0.05	> 0.06	
Potassium	Bloom / Leaf blade	0.70	> 0.80	1.40
	Bloom / Petiole	0.75-1.00*	> 1.20	
	Veraison / Leaf blade	0.60	> 0.70	1.20
	Veraison / Petiole	0.50-0.60*	> 0.70	

*Petioles were not as reliable as leaf blades in this research (mainly due to yearly variation).

Nitrogen guidelines based on obtaining YAN values of ~100 mg N/L without a yield reduction.

Phosphorus guidelines based on obtaining no yield reduction.

Potassium guidelines based on obtaining no drop in must pH.

However, petioles are less reliable than leaf blades for diagnosing N requirements (see *What Tissue Should I Test?* below). Results from any specific Pinot noir vineyard block may not align perfectly with guidelines proposed here; however, the overall vine responses to N supply should be similar. Therefore, if you monitor yield and canopy size and use these data together with your N tissue test results, you can develop a very accurate N tissue target to manage specific Pinot noir blocks.

Winemakers are often concerned that low YAN will lead to greater production of hydrogen sulfide and other sulfides during fermentation that can impart unpleasant rotten egg or cabbage aromas in wine (Bell and Henschke 2005). However, Pinot noir wines produced from this work where YAN levels ranged from 25-235 mg N/L showed that wine sulfides increased as must YAN increased, opposite of conventional wisdom. Therefore, concerns that sulfide production are due to low YAN may not be warranted. Yeast strain and other micronutrients (like vitamins) may play a bigger role in sulfide production (Wang et al. 2003, Ugliano et al. 2009).

Even though reducing N in the vineyard results in lower fruit YAN levels, numerous secondary metabolites that are important for wine quality are often increased by lowering vine N status (Bell and Henschke 2005). This was true in our NPK trials where anthocyanins, condensed tannins, and other phenolic acids increased in berries or wines with lower N status (Schreiner et al. 2014). This finding and other studies showing that red wine quality is improved by reducing vine N status (Bell and Robson 1999, Treeby et al. 2000, Pérez-Álvarez et al. 2013), provide evidence that Pinot noir quality may be enhanced when grown at a lower N status. Reducing vine N status can be accomplished by reducing N fertilizer inputs, or by other means such as increasing competitive vineyard floor vegetation and/or limiting tillage that increases soil N mineralization. My lab and colleagues are currently investigating how N availability affects wine composition in both Pinot noir and Chardonnay under commercial production conditions, and whether N additions in the winery can be used instead to boost YAN if that is beneficial for either variety.

Phosphorus. The lowest level of P that was used in the first NPK trial did not affect growth or yield of Pinot noir, so we included a treatment with zero-added P in the second trial. Vine growth and fruit yield were both reduced in the second study only after vines received no P for 3 years and this was repeated in year 4. Since yield and canopy growth were affected at the same level of P status, the use of P limitation to reduce vigorous canopies is not a good approach. Similar to N, P supply had the greatest impact on must P concentrations, which allowed us to test what the yeast assimilable phosphorus (or YAP) requirements might be. Fermentation rate was not altered by must P levels in our work after obtaining must P as low as 30 mg P/L, so the minimum YAP is below that level. Since berry chemistry and wine composition (including Brix, pH, TA, amino acids, anthocyanins, phenolics, aroma volatiles) were not consistently altered by P status in this research, P management appears to be a viticulture issue for Pinot noir with little expected impact on wine quality. Our tissue P guidelines for Pinot noir are based on yield which was

only reduced when leaf blade P at veraison was below 0.10% P (Table 1). This critical value for leaf blade P at veraison is very robust since vines in two different treatments in other years of the study reached leaf blade values equal to 0.10% P, and yield was not altered. A safe level of 0.11% P for leaf blade P at veraison is proposed to account for sampling and laboratory error. Critical values for leaf blade P at bloom and for petiole P at bloom and veraison are provided also (Table 1).

Potassium. The K levels applied in our first NPK trial had no effect on growth or yield of Pinot noir, so a zero-added K treatment was also used in our second trial. Yield was reduced only after receiving no K for 4 consecutive years. The yield loss due to K limitation was partly due to late bunch stem necrosis (LBSN). Late bunch stem necrosis can be caused by K deficiency that results in the accumulation of very high levels of putrescine in the rachis (Ruiz and Moyano 1994). About 40% of the fruit clusters from no K vines in year 4 had some degree of LBSN, while 8% of clusters had LBSN in the next highest K supply rate (20% K) which did not impact yield. Some LBSN symptoms also occurred in year 3 in no K vines but yield was not reduced. Vine growth based on pruning weights was reduced in year 4 in no K vines, and leaves from these vines displayed K deficiency symptoms in both years 3 and 4. We did not observe leaf symptoms in the 20% K vines at any time, even though minor LBSN symptoms occurred. However, our critical values for K tissue guidelines are not based on yield, canopy growth, or symptoms of LBSN because K limitation altered must pH **well before** yield or growth was reduced or fruit damage had occurred. Must pH was reduced in the no K vines in years 2, 3, and 4 as compared to the control vines. In addition, must pH was reduced in year 3 and 4 in vines receiving the next highest K level (20% K). A critical value for leaf blade K at veraison for Pinot noir producing similar yields as reported here is 0.60 % K with a safe value of 0.70% K (Table 1). For bloom, critical and safe leaf blade values are 0.10% higher than the corresponding veraison values. Petiole K values were slightly less reliable than leaf blade values in this trial (see below).

What Tissue Should I Test?

A regression analysis of the raw data from the second NPK trial showed that leaf blades were superior to petioles for diagnosing vine productivity and must YAN responses to varying N supply (Schreiner and Scagel 2017). The primary reason why petioles were not as good as leaf blades for N diagnosis was due to greater year to year variation in petiole N concentrations. This was true at bloom and at veraison. In addition, petioles had a lower dynamic range of N concentrations than leaf blades, and petioles had greater coefficients of variation. I have also found wider variation in petiole N compared to leaf blade N over years when resampling the same commercial vineyard blocks. Leaf blades were also better than petioles when assessing how N concentrations in each tissue responded to the different rates of N supplied based on mean values at each N rate studied. Leaf blade N had a more consistent response to the discrete N levels applied to vines than petiole N over time and resulted in a greater separation among different N treatment groups (Schreiner et al. 2018).

Regression analysis of the raw data revealed that leaf blades and petioles were equally good for diagnosing vine responses to varying P or K levels from our research. This was true because the dynamic range of the P and K values was greater in petioles than in leaf blades (particularly for K), but the coefficient of variation was also greater in the petioles. However, when we assessed how P and K concentrations in each tissue responded to different rates of these nutrients using mean treatment values, leaf blades were better than petioles. Leaf blades had more consistent responses to varying P and K rates than did petioles, resulting in a greater separation among the discrete P and K treatments. For these reasons, I recommend using leaf blades over petioles for routine nutrient testing in vineyards. Petioles have the advantage of being smaller, thus requiring more leaves (i.e. more vines) to be included in your samples which should reduce sampling bias and error. Petioles likely also have less spray deposits due to their smaller surface area which should reduce interference from chemicals sprayed on vines.

Both of these benefits of using petioles can be easily remedied by sampling leaf blades using a hole-punch and by washing the samples prior to analysis (which should be done by you or the testing lab anyway). Finally, it is intuitive that the leaves would be better for routine nutrient assessment, as the leaf blade is the metabolic workhorse of the canopy.

Further Reading

Schreiner RP, Lee J and Skinkis PA. 2013. [N, P, and K supply to Pinot noir grapevines: impact on vine nutrient status, growth, physiology, and yield](#). Am J Enol Vitic 64:26-38.

Schreiner RP, Scagel CF and Lee J. 2014. [N, P, and K supply to Pinot noir grapevines: impact on berry phenolics and free amino acids](#). Am J Enol Vitic 65:43-49.

Schreiner RP and Scagel CF. 2017. [Leaf blade versus petiole nutrient tests as predictors of nitrogen, phosphorus, and potassium status of 'Pinot Noir' grapevines](#). HortScience 52:174-184.

Schreiner RP, Osborne J and Skinkis PA. 2018. [Nitrogen requirements of Pinot noir based on growth parameters, must composition and fermentation behavior](#). Am J Enol Vitic 69:45-58.

Yuan F, Schreiner RP and Qian MC. 2018. [Soil nitrogen, phosphorus, and potassium alter \$\beta\$ -damascenone and other volatiles in Pinot noir berries](#). Am J Enol Vitic 69:157-166.

Literature Cited

Bell SJ and Robson A. 1999. Effect of nitrogen fertilization on growth, canopy density, and yield of *Vitis vinifera* L. cv. Cabernet Sauvignon. Am J Enol Vitic 50:351-358.

Bell SJ and Henschke PA. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Austr J Grape Wine Res 11:242-295.

Pérez-Álvarez EP, Martínez-Vidaurre JM, Martín I, García-Escudero E and Peregrina F. 2013. Relationships among soil nitrate nitrogen and nitrogen nutritional status, yield components, and must quality in semi-arid vineyards from Rioja AOC, Spain. Commun Soil Sci Plan 44:232-242.

- Ruiz RS and Moyano SA. 1994. The waterberry stem necrosis disorder in grapes and its relationship with elevated putrescine and low potassium content. *Agric Tec* 54:87-94.
- Stockert CM, Bisson LF, Adams DO and Smart DR. 2013. Nitrogen status and fermentation dynamics for Merlot on two rootstocks. *Am J Enol Vitic* 64:195-202.
- Treeby MT, Holzapfel BP, Pickering GJ and Friedrich CJ. 2000. Vineyard nitrogen supply and Shiraz grape and wine quality. *Acta Hort* 512:77-92.
- Ugliano M, Fredrizzi B, Siebert T, Travis B, Magno F, Versini G and Henschke PA. 2009. Effect of nitrogen supplementation and *Saccharomyces* species on hydrogen sulfide and other volatile sulfur compounds in Shiraz fermentation and wine. *J Agric Food Chem* 57:4948-4955.
- Wang XD, Bohlscheid JC and Edwards CG. 2003. Fermentative activity and production of volatile compounds by *Saccharomyces* grown in synthetic grape juice media deficient in assimilable nitrogen and/or pantothenic acid. *J Appl Microbiol* 94:349-359.
- During cold soak, grape must is held at cold temperatures (40-50°F) for several days prior to alcoholic fermentation (Casassa et al. 2015). Winemakers typically choose to employ this technique for two major reasons: to improve the color and/or mouthfeel of the wine, and/or to modify the flavor and aroma of the wine (Casassa et al. 2015). Research to date shows conflicting results as to the color enhancement benefits of cold soak-treated fermentation. For example, Poussier et al. (2003) found no increase in color or anthocyanins in Merlot wine with a 60 hr cold soak while Reynolds et al. (2001) reported higher anthocyanin content in wines produced from cold soaked grapes. This lack of consensus is likely due to variations in winemaking and viticulture practices, and differences in the grape varieties studied.
- While the impact of cold soak on color and phenolic content has received much attention, the effect on wine aroma and flavor is unclear. In particular, the role of microorganisms in the development of wine aromas is not well defined. Although *Saccharomyces* species typically dominate alcoholic fermentation, the presence and growth of non-*Saccharomyces* yeast, often in high numbers on the grapes, can potentially affect wine aroma (Zott et al. 2008). Furthermore, under cold soak conditions, the population of non-*Saccharomyces* yeast can rise to relatively high numbers due to their cold tolerance (Zott et al. 2008). This may enhance their impact of wine aroma and flavor. However, apart from elevated ethyl acetate production by *Hanseniaspora uvarum* (*Kloeckera apiculata*), the specific contribution of these yeasts to aromatic changes during cold soaking has not been reported.

Unravelling the Mysteries of Cold Soaking: The Role of Grape Microflora

Dr. James Osborne, Enology Extension Specialist & Associate Professor, OSU

Dr. Michael Qian, Professor, OSU

Dr. Elizabeth Tomasino, Associate Professor, OSU

Wine aroma is one of the most important yet complex components of wine quality, with hundreds of compounds contributing to wine aroma. These volatile compounds are derived from a number of sources including the grape, microbial flora, and biochemical changes due to aging process. They can also be impacted by grape variety, vineyard management practices, and winemaking procedures (Swiegers et al. 2005). In an increasingly competitive economic environment, winemakers are looking to utilize winemaking techniques to enhance the quality of their wines. One technique to modify red wine quality is a pre-fermentation cold maceration, commonly known as a cold soak.

One potential wine aroma impact of cold soaking is the increased release of grape-derived aroma compounds. Grape-derived aromas exist as both free volatile compounds and glycosidically-bound aroma precursors (Swiegers et al. 2005), and they can be liberated by acid- or enzymatic-hydrolysis to become aromatic. In red wines such as Pinot noir, the glycosidically-bound aroma compounds β -damascenone and β -ionone contribute positively

to wine aroma and are often described as rose, honey, and red fruit (Fang and Qian 2006). Cold soak is thought to increase the extraction of grape-derived aroma compounds, but little evidence exists that this occurs to an extent that would impact wine aroma. Yeast-produced β -glucosidase enzymes produced during cold soak may release the volatile free forms of the grape-derived compounds (Charoenchai et al. 1997). However, β -glucosidase activity is typically inhibited under high sugar conditions and cold temperatures that are common during cold soaking (Charoenchai et al. 1997). Finally, yeast activity during cold soaking may contribute to wine aroma through the production of aroma active compounds such as esters and higher alcohols. Small changes in the concentration of these compounds can have a significant impact on wine aroma, and cold soaking may alter the concentration of these compounds, especially esters.

While a number of studies report the impacts of yeast on wine aroma, many either were conducted in model systems, on white wines, and/or using non-sterilized grapes or must. Using non-sterilized grapes makes it difficult to draw conclusion for the specific contribution of each yeast strain or species, as background yeast on the grapes and winemaking equipment may impact the results. To address these issues, the Osborne and Qian Labs conducted a study (Hall et al. 2017) using Pinot noir grapes processed by high hydrostatic pressure (HHP) so that background microorganisms on the grapes could be eliminated while not affecting grape flavor and aroma compounds. This allowed specific non-*Saccharomyces* yeast to be added to the grape must during cold soak so the exact impact of these yeast could be determined. It also allowed the impact of the physical process of cold soaking to be analyzed independent of the action of the yeast, as a cold soak was conducted with no microorganisms present. Cold soaks were administered for 7 days at 46–48°F. The grape must was inoculated with either *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, *Lachancea thermotolerans*, or *Saccharomyces cerevisiae* isolate OSU-1. These yeasts had previously been isolated

from commercial Pinot noir lots undergoing cold soak and shown to grow under typical cold soak conditions. A treatment containing all of the yeasts was prepared (cold soak + all microorganisms) along with a cold soak treatment where no yeast was added (cold soak, no microorganisms). Wines were also produced without cold soaking.

Wine volatile aroma compounds were identified by gas chromatography and significant differences were noted between all treatments. Wines produced without a cold soak contained the highest overall ester content.

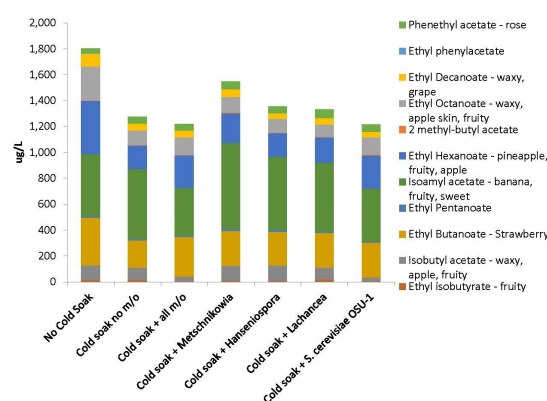


Figure 1. Total esters in Pinot noir wines produced with or without cold soaking and with the addition of various yeast.

Wines produced from cold soaks with various yeast species contained different concentrations of esters. For example, wines that underwent cold soak generally had lower concentrations of branch chain esters and lower concentrations of ethyl esters compared to the wines produced without cold soak (Figure 1). Interestingly, differences were also noted between the cold soak with all microorganisms treatment and the cold soak with no microorganisms treatment. This finding illustrates that both the physical process of the cold soak and the presence and growth of yeast can contribute to volatile aroma differences in wines. Aside from esters, there were some minor differences in grape-derived compounds, although cold soaking did not change the concentrations of β -damascenone and β -ionone, two grape-derived aroma compounds that contribute significantly to Pinot noir wine aroma (Fang and Qian 2006).

The major role of yeast present during cold soak was to alter the composition of esters in the final wine. Differences may have been due to either the production of esters by the yeast species or compositional changes caused by the growth of the yeast during the cold soak. The high population of yeast may have reduced the pool of amino acids available for ester production by *S. cerevisiae* during alcoholic fermentation, as ester production is impacted by nitrogen composition. Currently, the Osborne and Qian Labs are investigating whether yeast present during cold soak have a direct or indirect affect on wine ester composition. Volatile aroma compound concentration at the end of cold soaking and the end of alcoholic fermentation are being analyzed as well as the amino acid composition of musts after cold soak. These analyses will determine the direct effect of yeast during cold soak (production of esters) as well as the indirect effect (altering amino acid composition of the grape must impacting production of esters by *S. cerevisiae*).

While the analytical approach showed that cold soaking resulted in wines with different volatile aroma compositions, were those differences able to be perceived? To determine this, wines were assessed by a trained sensory panel under the guidance of Dr. Elizabeth Tomasino. Results are shown in Figure 2 where the principle component analysis shows how all the sensory descriptors used in the study relate (or correlate) to specific wines. Wines produced without a cold soak or with a cold soak with no microorganisms were grouped together and best characterized along the positive axis for F1. Specifically, no-cold soak wines had more floral and cooked, dark fruit aromas, while cold soak wines with no microorganisms had more oxidized and blackberry aromas. Wines produced from cold soaked grapes with the addition of *M. pulcherrima* and *L. thermotolerans* were characterized by black currant, strawberry, and volatile aromas, while wines produced from grapes cold soaked with all m/o and cold soaked with *S. cerevisiae* OSU-1 were characterized by reduced, mushroom, and confectionary fruit aromas.

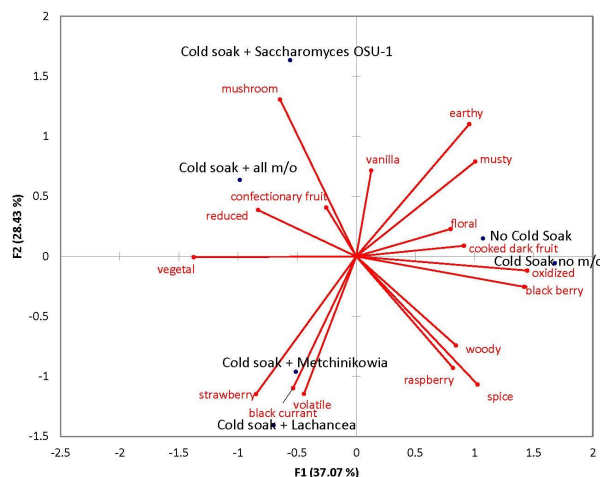


Figure 2. Principal component analysis of mean sensory data for Pinot noir wines produced with or without cold soaking and with the addition of various yeast.

These studies provide answers as to why cold soaking can result in changes in Pinot noir wine aroma and flavor. Both the physical process of cold soaking as well as the growth of yeast present during the cold soak can result in changes to wine volatile aroma compounds. The major differences were in ester and higher alcohol concentrations and were sufficient to result in wines that smelled different when assessed by a trained sensory panel. While cold soaking may be a beneficial winemaking technique for Pinot noir, it is not without risk. High concentrations of spoilage compounds such as acetic acid and ethyl acetate can be produced if excessive growth of spoilage yeast such as *Hanseniaspora uvarum* (*Kloeckera apiculata*) occurs. Current work in the Osborne lab is investigating how to manage cold soaks to minimize the risk of spoilage by *H. uvarum* while still gaining the potential benefits due to growth of other non-*Saccharomyces* yeast.

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Literature Cited

Casassa LF, Bolcato EA and Sari SE. 2015. Chemical, chromatic, and sensory attributes of 6 red wines produced with prefermentative cold soak. Food Chem 174:110-118.

Charoenchai C, Fleet GH, Henschke PA and Todd BEN. 1997. Screening of non-*Saccharomyces* wine yeasts for the presence of extracellular hydrolytic enzymes. *Aust J Grape Wine Res* 3:2-8.

Fang Y and Qian MC. 2006. Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *J Agric Food Chem* 54:8567-8573.

Hall H, Zhou Q, Qian MC and Osborne JP. 2016. Impact of yeast species present during pre-fermentation cold maceration of Pinot noir grapes on wine volatile aromas. *Am J Enol Vitic* 68:81-90.

Poussier M, Guilloux-Benatier M, Torres M, Heras E and Adrian M. 2003. Influence of different maceration techniques and microbial enzymatic activities on wine stilbene content. *Am J Enol Vitic* 54:261-266.

Reynolds A, Cliff M, Girard B and Kopp TG. 2001. Influence of fermentation temperature on composition and sensory properties of Semillon and Shiraz wines. *Am J Enol Vitic* 52: 235 – 240.

Swiegers JH, Bartowsky EJ, Henschke PA and Pretorius IS. 2005. Yeast and bacterial modulation of wine aroma and flavour. *Aust J Grape Wine Res* 11:139-173.

Zott K, Miot-Sertier C, Claisse O, Lonvaud-Funel A and Masneuf-Pomarede I. 2008. Dynamics and diversity of non-*Saccharomyces* yeasts during the early stages in winemaking. *Int J Food Microbiol* 125:197-203.

How do deficit irrigation and grapevine red blotch virus influence disease severity, water status, yield, and fruit composition?

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Grapevine red blotch disease (GRBD) is an economically important disease of grapevines caused by grapevine red blotch virus (GRBV). GRBD has been found in most major wine production regions across the United States.

Research groups are investigating various aspects of GRBD, with primary focus on vector biology and transmission or virology. Since there are no field-level management practices known for GRBD, many growers have removed young GRBV-infected vineyards. Information is necessary regarding the interactive effects of GRBD and vineyard management practices to determine whether the virus can be managed in lieu of vineyard removal.

GRBD negatively affects vine function, with economic losses related to reduced fruit yield and wine quality. The disease has been shown to reduce vegetative growth and canopy development, with an associated ripening delay and reductions in total soluble solids (TSS) of up to 5° Brix at harvest (Calvi 2011). There is also some evidence for a reduction in yield over time, with fewer cluster numbers per vine in some cultivars (Poojari et al. 2013). However, these results were from only a few studies and a limited number of cultivars in California and Washington.

Vineyards are irrigated in many of the regions most affected by GRBD due to limited summer rainfall. Supplemental irrigation effectively manages vine growth and fruit development in those semi-arid and arid regions. While moderate water deficits can advance ripening and improve fruit quality (Castellarin et al. 2007), severe water deficits can reduce photosynthesis, vegetative growth, and yield (Williams 2012, Williams et al. 2010a, 2010b). At veraison, water transport into the berries transitions from xylem to phloem, thus the advancement of ripening due to water deficits may also interact with GRBD given that the virus is phloem-limited. Therefore, water deficits may amplify or hasten the negative effects of GRBD on vine growth, productivity, and fruit quality.

Interactions between plant water deficits and disease development have been observed in earlier studies. Water use efficiency of two grape cultivars was significantly reduced with increasing infection by powdery mildew (Lakso et al. 1982). Similarly, a two-way interaction between the Pierce's disease pathogen (*Xylella fastidiosa*) and water

deficit has been observed at the molecular level (Choi et al. 2013). In this study, it was hypothesized that a similar interaction between water deficit and disease development occurs in GRBV-infected vines. Accordingly, a two-year study examining the impacts of GRBV infection and irrigation management was developed in southern Oregon. The results from this study are expected to provide information on managing water deficit to mitigate the effects of GRBD in vineyards.

Experimental Methods

The experiment was conducted in a commercial vineyard located in the Rogue Valley AVA near Jacksonville, Oregon. It was planted in 2009 to Pinot noir grafted onto Schwarzmann rootstock. Irrigation treatments were determined based on estimated crop evapotranspiration (ET_c) and imposed by adjusting the number of drip emitters per vine. Wet (W) treatments received water at 100% of estimated ET_c , while dry (D) treatments were irrigated at 66% ET_c . Vine disease status was confirmed in candidate vines (based on 2016 symptomology) for GRBV by PCR-based assays.

Symptom expression of GRBD was quantified in infected (RB+) and healthy (RB-) vines weekly, starting on date of first foliar symptom appearance until harvest. Vine water status (midday stem water potential; Ψ_{stem}) was measured at regular intervals throughout the season beginning at berry set. Just prior to commercial harvest, berry samples were collected, weighed, pressed, and juiced to measure total soluble solids (TSS), pH, and titratable acidity (TA). Skin and seed polyphenolics were determined by Harbertson-Adams assay. At harvest, cluster number per vine and total yield per vine were recorded. Cluster weight was estimated from vine yield and cluster count data, and berries per cluster estimated from berry weights.

Non-replicated, small-lot wines were made from the trial at the OSU pilot winery in Corvallis, Oregon. Wines were tasted blind by 42 individuals during the 4th Annual Southern Oregon Grape Symposium on 13 March 2018. Tasters did not know the order of the wines and were

only told that they were tasting “red blotch wines.” Tasters were anonymously polled on their preferences using clickers. Preference rankings were weighted such that 1st, 2nd, 3rd, and 4th place wines received 4, 3, 2, and 1 point, respectively for each taster. Then, each wine’s preference score was calculated as the number of points it received relative to the total number of points received by all four wines.

Summary of Results

Virus testing of nearly 100 candidate vines was conducted in the spring of 2017. Approximately 44% of vines tested positive for GRBV, while 56% tested negative. Although there was lower GRBV incidence in one area of the experiment, the distribution of GRBV was fairly uniform across all experimental blocks. In addition, there was a significant correlation between visual symptoms and positive virus test results, with 95% of 2016 symptomatic vines testing positive for GRBV in 2017, and 96% of the asymptomatic vines testing negative. The remaining 4% of the vines that were marked as asymptomatic in 2016 exhibited symptoms in 2017 and tested positive for the virus.

Vines in the W treatment received 163 gal. of applied water while D vines received 114 gal. of applied water over the course of the growing season. Irrigation commenced approximately two weeks after berry set, was applied weekly until lag phase, and then withheld until veraison. Following veraison, water was applied weekly for two more weeks when it was tapered off until harvest on 21 September. Vine water status was higher in W vines (Ψ_{stem} » -6 bars) compared to D vines (Ψ_{stem} » -8 bars) all season long. Diseased vines typically had a higher water status compared to healthy vines, but these differences were only statistically significant post-veraison. Notably, diseased vines had post-veraison Ψ_{stem} values that were approximately 1 to 1.3 bars higher (i.e. less negative; less stressed) compared to healthy vines.

The first foliar symptoms of GRBD were observed approximately two weeks before veraison on 25 July 2017. However, irrigation treatment did not influence the onset or the progression of foliar symptoms in diseased vines.

The rate of disease progress within diseased vines was slightly higher in D vines compared to W vines, but this difference was not statistically significant.

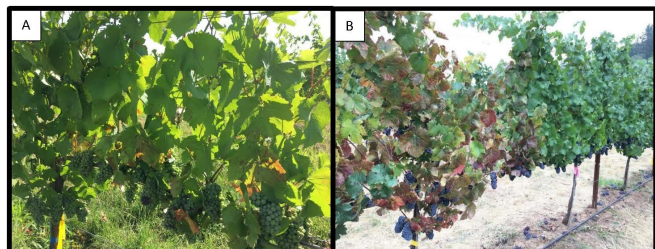


Figure 1. Red blotch disease progress over time in Pinot noir: A) First symptom on red blotch positive vine 07-25-2017; B) The entire vine showing symptoms and healthy vines next to the infected vine 09-12-2017, the blotches coalesce, and the leaf blades turn red between the margins.

GRBD raised vine water status throughout the growing season, but the differences in Ψ_{stem} between diseased and healthy vines were only significant after veraison, which in turn occurred after the onset of foliar symptoms. The relatively small differences in vine water status between diseased and healthy vines prior to veraison, coupled with a lack of differences in disease onset and severity suggests that different applied water amounts did not influence disease development. However, it should be noted that the water status of D vines was still high for most of the season ($\Psi_{\text{stem}} \geq -8$ bars), despite the 33% reduction in applied water amounts. Therefore, it is possible that water stress can influence disease onset and severity, but it may need to be more severe than was observed in the first year of this study.

Interestingly, yields were approximately 30% higher in diseased vines compared to healthy vines, and this was primarily due to 16% more, larger clusters, which had 10% more berries per cluster and 6-10% larger berries. The larger berries were likely due to the higher water status induced by GRBV infection, but it was interesting to find increases in other yield components as well. However, there were no significant differences in the Ravaz index among treatments (4.0-4.9).

Thus, any differences in fruit maturity were likely not confounded by differences in crop load and could only be attributed to the experimental treatments themselves.

Table 1. Response of fruit yield and maturity to treatments at harvest (21 September 2017). W-, D-, W+, and D+ treatments refer to well-watered/healthy, deficit irrigated/healthy, well-watered/red blotch, and deficit irrigated/red blotch, respectively. Values within a column followed by the same letter are not significantly different at $p < 0.05$.

Treatment	Yield (kg/vine)	Maturity			
		TSS (°Brix)	pH	TA (g/L)	
W+	6.7 b	22.7 ab	3.42 a	3.64 a	
W-	4.5 a	24.1 b	3.40 a	3.43 a	
D+	4.9 ab	21.4 a	3.46 a	3.48 a	
D-	4.3 a	22.7 ab	3.38 a	3.48 a	

Sugars were highest in control (i.e. well-watered and healthy; W-) vines, and there were no significant differences among treatments in either juice pH or TA (Table 1). Differences in TSS between diseased and healthy vines were only 1.4 °Brix, much smaller than previously reported in other studies from California. However, large differences among treatments were observed in secondary metabolites.

Table 2. Response of fruit polyphenolic concentration to treatments at harvest (21 September 2017). W-, D-, W+, and D+ treatments refer to well-watered/healthy, deficit irrigated/healthy, well-watered/red blotch, and deficit irrigated/red blotch, respectively. Anthocyanins are given in malvidin-3-glucoside equivalents (ME), while Tannins and Iron-reactive phenolics are given in catechin equivalents (CE). Values within a column followed by the same letter are not significantly different at $p < 0.05$.

Treatment	Anthocyanins (mg ME g ⁻¹ berry FW)	Tannins (mg CE g ⁻¹ berry FW)		Iron-reactive phenolics (mg CE g ⁻¹ berry FW)	
		Skin	Seed	Skin	Seed
W+	0.55 a	0.84 a	1.31 a	1.61 a	3.01 a
W-	0.67 a	0.84 a	1.67 a	1.85 ab	4.69 b
D+	0.59 a	0.80 a	1.36 a	1.85 ab	2.98 ab
D-	0.98 b	1.01 a	1.54 a	2.27 b	4.16 ab

There was an 18% reduction due to GRBV infection in well-watered vines, and a 40% reduction due to GRBV infection in deficit irrigated vines.

Additionally, iron-reactive phenolics (IRP) and tannins were reduced due to GRBD, though tannins not significantly. Apart from anthocyanins, disease status impacted polyphenolic composition of seeds more than the berry skins.

Small treatment differences in tannins coupled with the large differences in IRPs suggests that GRBV inhibits biosynthesis of non-tannin IRPs that are primarily composed of flavonols (e.g. quercetin) and flavan-3-ol monomers (e.g. catechin and epicatechin). This effect was more pronounced in seeds. Since these compounds are implicated in wine astringency and mouthfeel, this may offer some explanation as to why GRBV-infected fruit produces lower quality wines, especially when these effects are combined with reduced anthocyanin concentration in the skins.

In general, wine composition closely matched that of fruit composition, with concentrations of anthocyanins and tannins in wines highly correlating to analogous parameters in fruit. Overall, tasters generally preferred RB- wines across irrigation treatments.

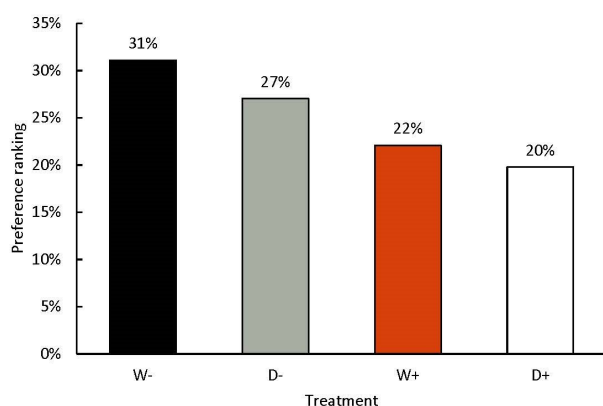


Figure 2. Weighted preference of 2017 wines tasted blind by 42 growers and winemakers on 13 Mar 2018 at the 4th Annual Southern Oregon Grape Symposium. W-, D-, W+, and D+ treatments refer to well-watered/healthy, deficit irrigated/healthy, well-watered/red blotch, and deficit irrigated/red blotch, respectively. Tasters were asked to rank wines in order of preference.

However, the differences in preference among all treatments were rather small (2-4%), and many tasters

reported that they preferred some characteristics of the RB+ wines or could not tell the difference. Interestingly, tasters preferred wines that were made from W vines compared to D vines.

Conclusions to date

These preliminary results suggest that keeping GRBV-infected vines well-watered may mitigate some of the negative effects of GRBD. Particularly, this may be advisable in order to promote vigor and avoid yield penalties associated with deficit irrigation practices. However, significant changes in berry secondary metabolism due to GRBV infection are likely not reversible under any irrigation management strategy. Ultimately, GRBD incidence may necessitate using infected fruit for different wine programs (e.g. rosé and/or sparkling) or blending with lots from healthy vineyards.

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Literature Cited

- Calvi BL. 2011. Effects Of Red-leaf Disease On Cabernet Sauvignon At The Oakville Experimental Vineyard And Mitigation By Harvest Delay And Crop Adjustment. Master's Thesis, University of California - Davis, Davis, CA.
- Castellarin SD, Matthews MA, Di Gaspero G and Gambetta GA. 2007. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227:101-12.
- Choi HK, Iandolino A, da Silva FG and Cook DR. 2013. Water Deficit Modulates the Response of *Vitis vinifera* to the Pierce's Disease Pathogen *Xylella fastidiosa*. *Molecular Plant-Microbe Interactions* 26:643-657.

Lakso AN, Pratt C, Pearson RC, Pool RM, Seem RC and Welser MJ. 1982. Photosynthesis, transpiration, and water use efficiency of mature grape leaves infected with *Uncinula necator* (Powdery mildew). *Phytopathology* 72:232-236.

Poojari S, Alabi OJ, Fofanov VY and Naidu RA. 2013. A Leafhopper-Transmissible DNA Virus with Novel Evolutionary Lineage in the Family *Geminiviridae* Implicated in Grapevine Redleaf Disease by Next-Generation Sequencing. *PLOS ONE* 11(1): e0147510.

Williams LE. 2012. Effects of applied water amounts at various fractions of evapotranspiration (ETc) on leaf gas exchange of Thompson Seedless grapevines. *Aust J Grape Wine Res* 18:100-108.

Williams LE, Grimes DW and Phene CJ. 2010a. The effects of applied water at various fractions of measured evapotranspiration on reproductive growth and water productivity of Thompson Seedless grapevines. *Irrigation Science* 28:233-243.

Williams LE, Grimes DW and Phene CJ. 2010b. The effects of applied water at various fractions of measured evapotranspiration on water relations and vegetative growth of Thompson Seedless grapevines. *Irrigation Science* 28:221-232.

Research Publications

Results of research conducted in viticulture and enology are published in peer-refereed academic journals, peer-reviewed reports, or books, which validates the scientific work of the authors. The following articles describe research conducted by members of the Oregon Wine Research Institute at Oregon State University.

Plant Pathology and Entomology

Ioriatti C, Guzzon R, Anfora G, Ghidoni F, Mazzoni V, Villegas TR, Dalton DT and Walton VM. 2018. [Drosophila suzukii \(Diptera: Drosophilidae\) Contributes to the Development of Sour Rot in Grape](#). *J Econ Entomol* 111:283-292.

Miller NE, Stoll R, Mahaffee WF and Neill TM. 2018. [Heavy particle transport in a trellised agricultural canopy during non-row-aligned winds](#). *Agric For Meteorol* 256-257:125-136 doi:10.1016/j.agrformet.2018.02.032.

Thiessen LD, Neill TM and Mahaffee WF. 2018. [Development of a quantitative loop-mediated isothermal amplification assay for the field detection of *Erysiphe necator*](#). *PeerJ* 6: doi:10.7717/peerj.4639.

Thiessen LD, Neill TM and Mahaffee WF. 2018. [Interruption and reduction of *Erysiphe necator* chasmothecia development utilizing fungicidal oil](#). *Plant Health Progress* 19:153-155 doi:10.1094/php-11-17-0070-rs.

Grapevine Nutrition

Schreiner RP, Osborne J and Skinkis PA. 2018. [Nitrogen requirements of Pinot Noir based on growth parameters, must composition and fermentation behavior](#). *Am J Enol Vitic* 69:45-58.

Yuan F, Schreiner RP and Qian MC. 2018. [Soil nitrogen, phosphorus, and potassium alter \$\beta\$ -damascenone and other volatiles in Pinot noir berries](#). *Am J Enol Vitic* 69:157-166.

Viticulture

Reeve AL, Skinkis PA, Vance AJ, McLaughlin KR, Tomasino E, Lee J and Tarara JM. 2018. [Vineyard floor management and cluster thinning inconsistently affect 'Pinot noir' crop load, berry composition, and wine quality](#). *HortScience* 53:318-328.

Extension Publications

Skinkis P. 2018. [Establishing a vineyard in Oregon: A quick-start resource guide](#). Oregon State University Extension Publishing EM8973-E.

Skinkis P, Pscheidt J, Moretti M, Peachey E, Walton V, KC A and Kaiser C. 2018. [Pest management guide for wine grapes in Oregon](#). Oregon State University Extension Publishing EM8413-E.