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VITICULTURE & ENOLOGY

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Welcome to the Spring 2019 Newsletter

This edition 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. Patty Skinkis, OSU Viticulture Extension Specialist and Professor, opens the newsletter with an article on canopy yield management. Dr. James Osborne, OSU Enology Extension Specialist and Associate Professor, discusses the importance of effective microbial monitoring in preventing microbial spoilage. Lastly, Sarah Lowder, OSU Graduate Research Assistant, along with Dr. Walt Mahaffee, Research Plant Pathologist, USDA-ARS, provide an article on techniques to monitor Qol fungicide resistant grape powdery mildew.

This issue is posted online at the OWRI website https://owri.oregonstate.edu/owri/extension-resources/owri-newsletters. Learn more about our research and engage with the core faculty here.

Cheers,
The OWRI Team

Crop thinning research: What are the limits?

Dr. Patty Skinkis, Viticulture Extension Specialist and Professor, OSU

The yield-quality paradigm has long driven vineyard management decision-making, with growers focusing on the level of cluster thinning needed to reach target yields. The general thought is that reducing yield will improve ripeness and quality, allowing the remaining fruit to accumulate desirable aroma, flavor, and color compounds. Many winemakers cite "concentration" as a reason for cluster thinning grapevines for the optimization of quality. Research literature notes improved total soluble solids (TSS, Brix) accumulation with cluster thinning under circumstances of over-cropping, as vines have reduced ability to ripen fruit beyond a certain crop level (Kliewer and Dokoozlian 2005, Kliewer and Weaver 1971). Likewise, cluster thinning increases TSS accumulation under certain stress conditions, including limitations on water (Gamero et al. 2014) or nutrients (Reeve et al. 2016). However, other studies show minor to no impact of cluster thinning on basic fruit ripeness or composition (Bowen et al. 2011, Keller 2005, Brasher 2002).

In this issue:

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Suprisingly little scientific evidence exists to indicate that cluster thinning leads to greater or improved concentrations of aroma, flavor, or mouthfeel compounds. In fact, some studies show advanced ripeness (TSS, pH, and titratable acidity (TA)) does not always equate to improved wine quality (Bravdo et al. 1985).

Over the past seven years (2012-2018), I led a team of researchers at Oregon State University and more than 20 vineyard and winery companies in Oregon to scientifically evaluate how cluster thinning and yield influence fruit ripeness and wine quality for Pinot noir. The goal was to determine what level of cluster thinning enhances quality under different vineyard conditions and seasons. The study was conducted in one to three acre vineyard blocks in commercial vineyards with two to five thinning treatments applied. The thinning treatments were applied at lag phase of berry development to whole vine rows in a randomized complete block design with a minimum of three field replicates at each site. Most companies applied thinning treatments based on a defined number of clusters per shoot (e.g. 0.5, 1, 1.5, and 2 clusters per shoot), with the majority of collaborators comparing 1 and 2 clusters per shoot. A few collaborators cluster thinned based on a target number of tons per acre. A full crop (no thinning) treatment was encouraged for comparison whenever possible, and 49% of participants included full crop treatment in their trials. The same cluster thinning treatments were applied in each year of the study to the same vine rows to evaluate long-term effects.

Data were collected on various vine growth measures to determine how sustained high or low crop levels were affecting vine growth, nutrient status, and yield productivity in addition to fruit composition. Data were collected on ten reference vines within each experimental plot. Vineyard data analysis included pre-bloom fruitfulness counts (number of inflorescences per shoot), lag phase cluster counts and weights, véraison leaf blade and petiole tissue sampling for macro- and micronutrient analyses, whole vine yields at harvest, and dormant pruning weights following each crop year. Fruit was sampled at harvest, using one harvest date for all treatments in most vineyards and years. There were a few vineyards where differential harvest was conducted, with the lower crop level being harvested before the heavier crop levels, but it was the exception rather than the norm. Fruit was analyzed for basic ripeness, including total soluble solids (TSS), pH, and titratable acidity (TA) at harvest. For the first five years of the trial (2012-2016), fruit was analyzed by ETS Labs using the grape juice and rapid phenolic panels. Since 2017, fruit was analyzed by the Skinkis Lab for basic ripeness, yeast assimilable nitrogen, and total concentrations of anthocyanin, tannins, and phenolics. Wines from the project were produced by participating wineries (minimum of 1.5 ton fermenters per crop level) following standard winemaking protocols. Wines were bottled and aged for two years until they underwent descriptive sensory analysis by a professional winemaker panel. Results of the vineyard and fruit aspects of the project are reported here for 2018 with comparisons to the prior six-year period.

Eleven Pinot noir vineyards were involved in the project during 2018, all located within the Willamette Valley (Table 1). The majority were in the project for more than five years. The mean harvest yield across all sites and crop levels for 2018 was 1.04 lb/ft (Figure 1), which is higher than the 7-year average of 0.95 lb/ft. The 2018 season had yields that were similar to 2014 and 2015. Mean yields across all cluster thinning treatments and sites were over 1.0 lb/ft in 2018 and most years of the study (Figure 1). High yields in recent years are likely due to high fruitfulness and larger cluster weights. The fruitfulness in spring 2018 was 1.7 inflorescences per shoot, which was statistically similar to 2015 to 2017, which had 1.6 to 1.7 inflorescences per shoot. Fruitfulness has been gradually increasing over the duration of the study since 2013 (1.4 inflorescences per shoot). While small increases in fruitfulness per shoot may seem negligible, a fraction of shoot fruitfulness can lead to significant differences on a tons per acre basis. The mean cluster weight in 2018 was 120 g, which was similar to 2015 (136 g) and 2017 (130 g). Cluster weights were less than 100 g during the lowest yielding years (2012, 2013, and 2016) and 108 g in 2014.

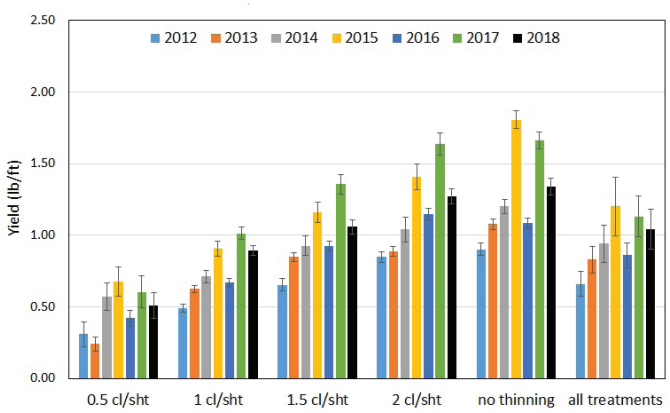

Figure 1. Mean (+ SE) yield measured across vineyard sites during 2012-2018 with different crop thinning treatments, including 0.5 clusters/shoot, 1 cluster/shoot, 1.5 clusters/shoot, 2 clusters/shoot, and no cluster thinning. All treatments includes the mean yield from all cluster thinning treatments and vineyards during each year.

Table 1. Site details for collaborating vineyards during the 2018 crop year.

Vineyard ID	American Viticultural Area (AVA)	Years in study	Year planted	Pinot noir clone	Rootstock	Planting Density (vines/A)
7134	Chehalem Mountains	7	2001	Pommard	3309C	1089
4297	Chehalem Mountains	1	2009	777	Riparia Gloire	968
3280	Eola-Amity Hills	8	1999	Dijon 114	Schwarzmann	1936
2115	Eola-Amity Hills	2	2007	Wadenswil	3309C	1210
2478	Eola-Amity Hills	6	2002	Pommard	101-14	1245
7340	Eola-Amity Hills	7	2001	Pommard	3309C	806
5234	Eola-Amity Hills	7	2004	667	Riparia Gloire	2530
6810	Willamette Valley	6	1983	Pommard	own-rooted	605
3370	Willamette Valley	6	2009	Pommard	3309C	1815
9927	Yamhill-Carlton	3	2005	115	101-14	2530
3836	Yamhill-Carlton	7	2001	777	3309C	2024

There were fewer differences in fruit composition during 2018 than observed in most other years of the study. Only 55% of the vineyards had a cluster thinning treatment difference in one or more fruit composition parameters, compared to an average of 75% of vineyards having some crop level effect in the six years prior. Only two sites in 2018 showed higher TSS with cluster thinning. Similarly, only two sites had higher total anthocyanin with cluster thinning (Figure 2, Table 2). However, the same sites did not always have differences in both TSS and total anthocyanin. In fact, only one of the sites showed both higher TSS and total anthocyanins with the more cluster thinning. Across the seven-year period, cluster thinning affected TSS and anthocyanin more often than other fruit parameters measured (Figure 2), but it was at or less than 38% of vineyards for TSS and 25% of the vineyards for total anthocyanin. Furthermore, there was no consistency in which vineyards showed cluster thinning effects each year. When there were statistical differences in fruit composition, there was often less difference than expected given the percent of yield reduction. When a difference was observed, it normally was for the lowest crop levels (1 cluster/shoot or less) compared to higher crop levels (No thinning, 2 clusters/shoot, and 1.5 clusters/shoot); the 1 cluster per shoot treatments had on average ~40% yield reduction compared to full crop (no thinning). The differences in fruit composition found in three sites during 2018 are shown to exemplify the relatively small magnitude of difference found in this study (Table 2). When observing means presented in this trial, it is tempting to see differences that were not found by statistics, as variance measures are not easily shown without cluttering data tables. In many cases, there was enough variance in the fruit coming from the different crop level treatments that statistical differences could not be found. Because of the relatively large plot size in these studies, this outcome is not uncommon, and likely reflects the true variance in the population found on most sites.

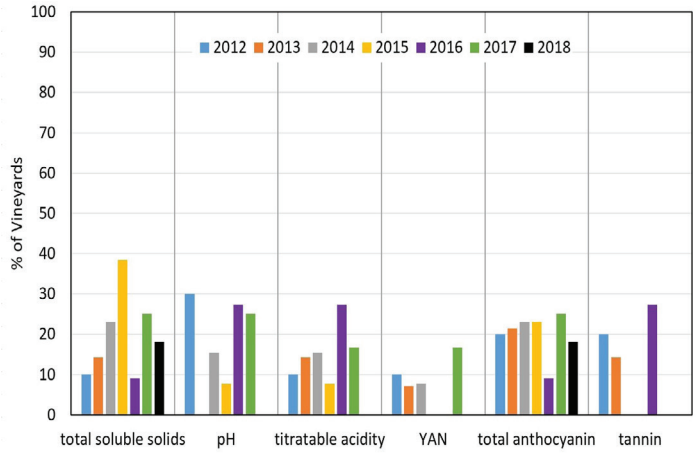

Figure 2. Percentage of all vineyards in that year that had statistical differences in fruit composition based on cluster thinning treatments (by analysis of variance). YAN-yeast assimilable nitrogen concentration (note that 2018 data are not included as the assays are in progress as of this reporting). Tannin – total tannin concentration of whole berries. Total anthocyanin – total tannin concentration of whole berries. Other fruit composition parameters were measured, but only data for those parameters collected across all years are shown here.

Table 2. Yield and fruit composition parameters at harvest for three vineyards in 2018.

Vineyard ID	Treatment	yield (lb/ft)	TSS (°Brix)	pH	TA (g/L)	total anthocyanin (mg/g)
3280	1 cluster/shoot	0.59 b	23.3 b	3.1	8.1	0.75
	No thinning	1.12 a	23.8 a	3.1	8.2	0.54
	p	0.0228	0.0377*	ns	ns	ns
6810	1 cluster/shoot	0.63 b	22.6 ab	3.1	9.0	0.70 a
	Half crop	0.51 b	23.1 a	3.1	9.1	0.72 a
	No thinning	0.86 a	21.3 b	3.0	9.5	0.53 b
	p	0.0120	0.0285	ns	ns	0.0123
3370	1 cluster/shoot	0.38 b	22.6	3.2	4.9	0.75 a
	1.5 clusters/shoot	0.61 a	21.2	3.1	5.1	0.57 b
	2 clusters/shoot	0.63 a	20.3	3.1	5.2	0.56 b
	No thinning	0.68 a	20.9	3.1	5.2	0.58 b
	p	0.01	ns	ns	ns	0.0067

Means presented. Yield presented in pounds per foot of vine row occupied by the vine canopy. TSS – total soluble solids, TA – titratable acidity in tartaric acid equivalents, total anthocyanin concentration shown in mg/g berry, determined using the pH-differential method. Different letters following the means indicates a statistical difference in those means based on Tukey's Honestly Significant Difference test at α=0.05. ns – not significant based on analysis of variance with $p > 0.05$. *Vineyard 3280 harvested the 1 cluster/shoot treatment on 27 Sept 2018 and No thinning on 7 Oct 2018. However, the sample comparison of the two thinning treatments on 27 Sept 2018 show no differences for TSS, pH, and TA.

Crop load, as defined by the yield to pruning weight ratio, differed by treatment and site in 2018. Crop loads ranged from 2.8 to 7.3 across all vineyard sites, and were statistically different by crop level at most sites. Crop load was the highest of all years of the study (Figure 3), likely due to the lower pruning weights measured at all sites in 2018.

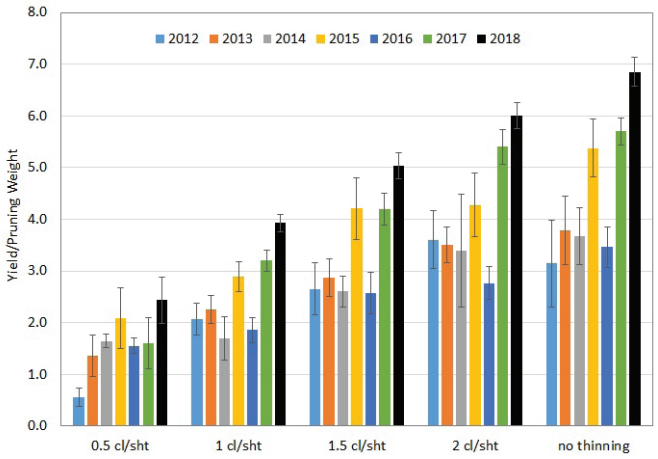

Figure 3. Mean (± SE) crop load (yield/pruning weight) by cluster thinning treatment and year from all vineyard sites during 2012-2018. Cluster thinning treatments are listed by number of clusters per shoot (cl/sht) and no cluster thinning. The 0.5 cl/sht treatment is defined by 1-0-1-0 thinning pattern per consecutive shoot on a vine, and the 1.5 cl/sht treatment is defined by the 1-2-1-2 thinning pattern per consecutive shoot on a vine.

Despite having one of the highest crop loads for the study during 2018, there were fewer differences in fruit composition reported at harvest than any year prior, suggesting that a higher crop load did not greatly impact fruit ripening. However, further regression analyses continue with these data to understand the relationships between yield, vine size (pruning weight), crop load (yield/PW) and other parameters.

The higher sustained crop levels in this study had relatively little impact on vine size and nutrient status compared to the lower crop level treatments over the years. Surprisingly, there have been no differences in fruitfulness, dormant pruning weights, or vine nutrient status that would suggest that full crop (no thinning) or higher crop level vines (2 clusters/shoot) are over-cropped and causing physiological stress. However, a few vineyards have recently shown lower pruning weights with higher crop levels, including two sites (6810 and 9927) in 2018. However, one of the vineyards (6810) had low productivity with 0.86 lb/ft for their full crop vines (Table 2) compared with most vineyards that had more than 1 lb/ft for their full crop or two clusters per shoot treatments in 2018. Furthermore, this was the only site that showed reduced fruit ripening based on lower TSS with higher crop level (Table 2). The lack differences in fruit ripening with higher crop levels in 2018 is another indicator that carbohydrate assimilation is not lacking given the vineyard yields and canopy sizes represented in this study.

There were treatment differences in leaf blade and/or petiole nutrient concentration at most vineyards in 2018 based on véraison tissue samples, but the differences were not consistent across sites or tissues within a site, and were rarely different for the macronutrients (nitrogen, phosphorus, and potassium). There were some sites with a crop thinning effect on manganese, magnesium, zinc, and calcium. When there was a difference in a specific nutrient concentration, the higher crop level (no thin or 2 clusters/shoot) was normally lower in that nutrient concentration than the more thinned treatments (e.g. 1 cluster/shoot). However, in a few cases, nutrient concentration was higher in the higher yielding treatments. It should be noted that no differential nutrient management practices were used

in this study to offset potential differences in crop level, as collaborators maintain uniform vineyard management practices across their vineyard block. These data suggest that maintaining a heavier crop has not significantly affected vine nutrient status.

So, what are Pinot noir yield limits for Oregon's Willamette Valley? Results to date suggest that yield limits are flexible and are relative to the vineyard site. This study encompassed vineyards with high and low productive capacity (yield), and vine response to yield has been relatively consistent--there are few to no differences in fruit composition in most years of the study. When there were differences, it was in TSS or total anthocyanins, but these differences were inconsistent over the years. The lack of differences in fruit composition was reflected in the lack of sensory differences in the wines produced. In most cases, there were no differences in wine descriptive analysis by crop level when considered within or across vineyards. Some in-house wine sensory results received from collaborating wineries indicates that there are differences perceived in wines from different cluster thinning treatments, but it is hard to determine clear preferences and they report that different crop levels can produce good quality wine. Often wineries did not prefer wines from the highest or the lowest yields, and a modest cluster thinning treatment was most often preferred (e.g. 1.5 clusters/shoot). When considering how to best target yields during cluster thinning, it is important to consider the productive capacity and heat units of a given year. The past seven years of the study have been warmer than the long-term average. Managing yield under these changing conditions require a renewed view on yield targets, focusing more on what each vineyard can produce based on the vine fruitfulness and canopy size. This requires a shift to thinking of yield in pounds per linear foot rather than tonnage per acre, as it becomes easier to compare performance from vineyard to vineyard. The renewed vision on yield management will also likely help improve production economics.

This work would not be possible without the dedication of the industry collaborators to the duty of carrying out the research on-farm and in the winery. Study co-principal investigators include Dr. James Osborne, Dr. Elizabeth Tomasino, and Dr. Katie McLaughlin of OSU, and Dr. R. Paul Schreiner, USDA-ARS. This study has been funded in part by research grants from the American Vineyard Foundation, the Oregon Wine Board, and the Agriculture Research Foundation. Funds were also provided by the Oregon Wine Research Institute pilot project program. ETS Labs and Fruit Growers Lab donated fruit composition and nutrient analysis services, respectively.

Literature cited

Bowen P, Bogdanoff C, Usher K, Estergaard B, Watson M. 2011. Effects of irrigation and crop load on leaf gas exchange and fruit composition in red winegrapes grown on a loamy sand. Am J Enol Vitic 62:9-22.

Brasher EW. 2002. Effect of crop level on yield components, fruit and wine composition, and wood carbohydrate reserves of Pinot noir grapes. Thesis, Oregon State University, Corvallis.

Bravdo B, Hepner Y, Loinger C, Cohen S, Tabacman H. 1985. Effect of crop level and crop load on growth, yield, must and wine composition, and quality of Cabernet Sauvignon. Am J Enol Vitic 36:125–131.

Gamero E, Moreno D, Talaverano I, Prieto MH, Guerra MT, Valdés ME. 2014. Effects of irrigation and cluster thinning on Tempranillo grape and wine composition. S Afr J Enol Vitic 35:196–204.

Keller M, Mills LJ, Wample RL, Spayd SE. 2005. Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. Am J Enol Vitic 56:91–103.

Kliewer WM,Dokoozlian NK. 2005. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. Am J Enol Vitic 56:170-181.

Kliewer WM, Weaver RJ. 1971. Effect of crop level and leaf area on growth, composition, and coloration of `Tokay' grapes. Am J Enol Vitic 22:172–177.

Reeve AL, Skinkis PA, Vance AJ, Lee J, Tarara JM. 2016. Vineyard floor management influences 'Pinot noir' vine growth and productivity more than cluster thinning. HortScience 51:1233–1244.

Effective microbial monitoring is key to preventing microbial spoilage

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

The production of wine involves a myriad of different microorganisms that have a significant effect on wine quality. Many microbes, such as *Saccharomyces cerevisiae* and *Oenococcus oeni*, are fundamental to the production of quality wine while others can cause significant spoilage. The management of these microorganisms from harvest to bottle is key to the production of high-quality wines. Unlike many other food systems, there is minimal effort given to fully eliminating all microorganisms from the raw ingredients of a wine. In fact, throughout wine production, the only stage where complete removal of microorganisms occurs is sterile filtration prior to bottling. At every prior step, there is the understanding that the wine contains living microorganisms. The growth of these microorganisms needs to be managed by the winemaker so that the growth of desirable microbes at key times (such as during the alcoholic and malolactic fermentation) is encouraged while the growth of spoilage microbes is discouraged. An important part of this process is to understand which microbial species are present, when and what their populations are, and what steps can be taken to manage their growth. Developing a robust microbial monitoring program as part of a larger quality control plan for the winery will help in the proactive prevention of microbial spoilage rather than merely reacting to spoilage issues when or after they occur.

When developing a microbial monitoring plan, first considering what the goals of testing will be for your winery. Why are you conducting microbial testing? How will this information be used? Will decision-making be informed by this testing? Every winery will likely have different goals that are specific for their facility and wine production practices. Once this has been established, you can move on to identifying the key points during your winemaking process where knowing microbial populations and composition will be most effective. Analysis of any type, whether chemical or microbial, costs time and money, so identifying the key stages where you will commit effort and resources is important. The reason for conducting a particular analysis at a particular time should be tied to potential action. For example, while it might be interesting to know what the population of *S. cerevisiae* is at the end of alcoholic fermentation (AF), this information is unlikely to prompt any particular winemaking decisions. On the other hand, microbial analysis of grape must at the beginning of a cold soak will provide information that could determine the length of cold soaking, sulfur dioxide rates, or inoculation strategy. Determining the critical points where you will perform analysis will be winery and wine- specific. Resources available for testing, either in-house testing or external lab testing, will differ between wineries as will red winemaking vs. white winemaking. However, there are a few general time-points during wine production where microbial testing is commonly performed.

The first critical point where microbial testing is useful is after fruit processing. Analysis of grape must and juice at this point will provide information on initial microbial populations and in combination with juice/must chemistry, the risk of microbial spoilage pre- and early fermentation can be assessed. The risk of microbial spoilage should always be considered in the context of chemical parameters. Of most importance is pH as this will affect the growth of microorganisms as well as the effectiveness of SO_2. For example, low pH grape must will present a lower risk for microbial spoilage during cold soaking than a high pH must with the same microbial load. The initial SO_2 added will also be more effective in the low pH must at reducing populations of naturally present microorganisms. Information about the microbial load coming in on the grapes may also determine management strategies post-AF. For example, a high population of *Acetobacter* pre-fermentation may not cause issues during AF, but elevated populations of this bacteria in the wine could lead to spoilage issues during aging. If you are aware that a particular grape lot has high *Acetobacter* pre-AF then you may change your topping and/or SO_2 checks during barrel aging to ensure these bacteria do not have a chance to proliferate.

A second critical point where microbial testing can be

effective is post-AF prior to malolactic fermentation (MLF). At this stage, wine can be very vulnerable to a number of spoilage microorganisms. The wine is likely still warm from fermentation, contains little to no free SO_2 and is at risk for spoilage by wine lactic acid bacteria and *Brettanomyces*. Knowing what the microbial populations are at this point may help you make decisions regarding MLF such as inoculation strategies, temperature of storage, and whether to conduct MLF in barrel or tank. It may also be helpful to know what your microbial populations are at Post-MLF if the wine will be aged for an extended time in barrels. Regular chemical testing during wine aging can also indicate microbial spoilage problems and prompt more specific microbial testing. For example, abnormally quick depletion of free SO_2 may indicate the production of acetaldehyde by spoilage microbes such as *Acetobacter* and/or film-forming yeast. A spike in volatile acidity can also indicate oxidative spoilage.

The most common point during winemaking where microbial testing is conducted is pre-bottling. Once the wine is in the bottle, it is very difficult to correct any microbial issues. The worst-case scenario is that a consumer is the one who discovers the microbial issues with the wine. Microbial testing will help inform the winemaker what steps need to be taken to ensure the microbial stability of the wine. In many cases, this means sterile filtration using a membrane filter. Results from pre-bottling microbial testing take on a different significance than testing earlier in the process. For example, a low number of *Brettanomyces bruxellensis* (10-50 cfu/mL) in wine early during barrel aging may prompt a different response than the same population pre-bottling. Both scenarios involve recognizing and evaluating risk. However, at an earlier stage in the winemaking process, the winemaker has more options available and may weigh the risks differently. They may decide that the wine is at low risk of further microbial spoilage due to low pH, low cellar temperature, and free SO_2 concentrations. The winemaker may choose to monitor the particular lot more frequently and hold off any further intervention at this stage. However, just prior to bottling a wait and see approach is no longer acceptable and so intervention is required.

When assessing the risk of microbial spoilage the chemistry of the wine should also be considered. Certain wine chemistries will inherently present a larger risk for spoilage. Consider residual sugar, residual malic acid, pH, molecular SO_2, and the history of the wine (has the wine been problematic during production, prior microbial issues, etc.). As a side note, if your goal is to completely remove microbes from your wine you must use sterile membrane filtration. Cross-flow filtration is not the same as sterile filtration, and cross-flowed wine may still contain microorganisms.

The time and effort spent on developing a comprehensive and robust microbial testing plan can all be wasted if you do not pay careful attention to how samples are taken for analysis. If careless, not only will your analysis be inaccurate, it may lead you to either making unnecessary interventions or doing nothing when your wine is actually at high risk of spoilage. The two most common errors made when sampling for microbial analysis are taking a non-representative sample and contamination of the sample. Taking a representative sample of grape must can be difficult due to the challenge of homogenizing a container of processed grapes. Combining multiple samples from various depths of the tank will help minimize some of the variability that exists within the container. However, you should keep in mind that the microbial populations measured in a tank of grapes might not be as accurate as those measured in a tank of wine that can be mixed to ensure homogeneity prior to sampling. Mixing is particularly important when taking a sample for microbial analysis as microbes may stratify within a tank or barrel. Larger microorganisms such as *Brettanomyces* (Figure 1) tend to settle to the bottom of a tank or barrel while aerobic microorganisms such as *Acetobacter* will be in higher populations near the surface of the wine where there is a higher concentration of oxygen. Ideally, the tank or barrel will be mixed before sampling. Sampling an hour or two after filling a tank or barrel will also ensure a representative sample. If mixing or stirring a tank or barrel is not an option, then sample from the top, middle, and bottom of the tank or barrel and make a composite sample.

Remember also that microbial populations will vary from barrel to barrel so plan sampling accordingly. If you are monitoring microbial populations over time, make sure to sample from the same barrels each time so that changes in populations can be determined. The goal is to have consistent and representative samples.

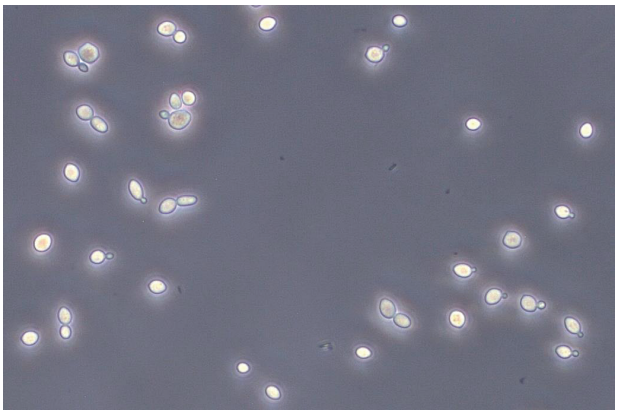

Figure 1. *Brettanomyces bruxellensis* tends to settle to the bottom of tanks and barrels over time. Mixing prior to sampling is needed to ensure a representative sample is taken.

Contamination of samples during the sampling process can lead to false positives or overestimation of the true population. An example of this would be when sampling a barrel you scrape the barrel thief against the inside of the barrel bunghole. *Acetobacter* are often in high concentrations around the barrel bunghole as they are aerobic microorganisms. Scraping the wine thief against the bunghole will likely contaminate the wine thief with a high population of *Acetobacter* that will then be transferred into the wine sample. Results of the analysis will incorrectly indicate that the wine has a high population of *Acetobacter* when in fact the wine may not. Care should also be taken when sampling from a tank using the sampling valve (Figure 2). If the valve was not been cleaned and sanitized properly, it is likely to contain residual wine. This wine will have a high population of aerobic microorganisms such as *Acetobacter* and so wine samples taken from the valve will be contaminated. Valves must be cleaned and sanitized before and after use (70% alcohol or SO_2/citric solution). In addition, several volumes of wine should also be flushed through the valve before taking a sample.

Figure 2. When collecting a sample from a tank-sampling valve, flush several volumes of wine through the valve before taking a sample to prevent contamination. Sanitize the valve before and after use.

In summary, developing a robust and effective microbial monitoring plan is key to the prevention of wine microbial spoilage. Every wineries plan will differ but there are some common strategies to take when developing one:

- Identify the goals for your testing program. What do you specifically want to achieve?
- Based on these goals, identify the critical points in your winemaking process where you need to know the microbial populations.
- Determine which testing methods to use at each critical point.
- Link testing with potential action. Establish thresholds or ranges that prompt action. These may differ depending on the stage of production and wine chemistry.
- Establish consistent and representative sampling protocols that minimize the risk of sample contamination.
- Review testing program periodically and consider whether it is achieving the goals that you have set out or if adjustments need to be made.

Literature cited

ETS laboratories. Collecting samples for microbial analysis. https://www.etslabs.com/library/41

Osborne JP. 2010. Advances in microbiological quality control. *In* Managing Wine Quality Volume 1: Viticulture and wine quality. AG Reynolds (ed.), pp. 162-188. Woodhead Publishing, United Kingdom.

Fits like a glove: Improving sampling techniques to monitor QoI fungicide resistant grape powdery mildew

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Dr. Walt Mahaffee, Research Plant Pathologist, USDA-ARS

Introduction

Grape powdery mildew (GPM), caused by the ascomycete fungus *Erysiphe necator*, is the most severe and widespread disease in western vineyards and can significantly reduce yields and quality if left uncontrolled (Gadoury et al. 2012). Since almost all commercially grown grape cultivars are susceptible to *E. necator*, disease management relies predominately on numerous fungicide applications. (Cadle-Davidson et al. 2011). However, the widespread and frequent use of quinone outside inhibitors (QoIs; FRAC Group 11) has led to the appearance of QoI resistance in most US grape production regions, thus causing losses in yields and quality from failed disease control (Miles et al. 2012, Wong and Wilcox, 2002). Although the occurrence of fungicide resistance is well documented in most of the U.S., the incidence, frequency, and distribution of fungicide resistant strains of *E. necator* in the Western U.S. are still largely unknown.

Monitoring fungicide resistance of *E. necator* was traditionally limited by time and costs of conducting intensive germination bioassays. However, a single mutation, G143A, in the cytochrome b gene in the mitochondria can cause complete resistance to the FRAC 11 group and, so far, is the only mutation we have found in *E. necator*. We used this knowledge to develop a competitive Taqman qPCR assay to detect QoI resistant *E. necator* and expedite the monitoring process. It was then unclear how to sample vineyards efficiently and accurately to assess resistance in a field population due to the logistical difficulty of sampling a microscopic, aerially dispersed fungal pathogen over large geographic areas (Falacy et al. 2007, Thiessen et al. 2017). So, our group, including Tara Neill of USDA-ARS, Ioannis Stergiopoulos of UC Davis, Tim Miles of MSU, and Michelle Moyer of WSU, has been working to develop methods that rapidly and accurately assess a vineyard for fungicide resistance so that growers have a better understanding of the risk of using different fungicides.

Methods

Resistance monitoring kits (Figure 1) consisting of a 2 ml micro-centrifuge tube, single-use forceps, and two white adhesive cryo-labels ("ToughSpots") in a labeled zip-seal bag (Thiessen et al. 2019) were distributed to collaborators throughout California, Oregon, Washington, British Columbia, Michigan, and Georgia in 2018 (Figure 2).

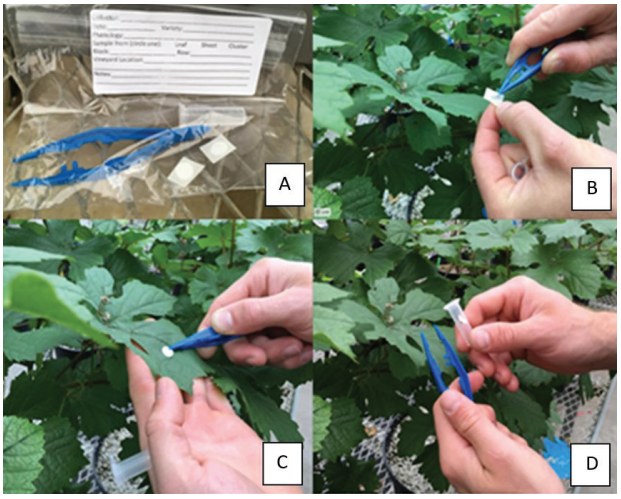

Figure 1. *Erysiphe necator* ToughSpot sampling kit and collection process: A – the assembled kit consisting of a 2 ml micro-centrifuge tube, single-use forceps, and two white adhesive cryo-labels ("ToughSpots") in a labeled zip-seal bag; B – Forceps used to pick up ToughSpot sticker; C – ToughSpot placed directly on fungal colony; D – ToughSpot inserted into tube and back into the labeled bag.

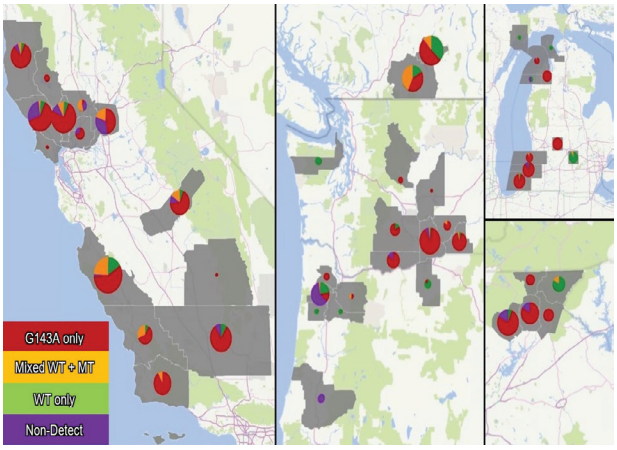

Figure 2. Schematics showing the relative proportion of ToughSpot samples that contained the QoI resistance G143A allele by county. Diameter of pie graph indicates number of samples relative to each location with 1403 samples represented.

ToughSpot kits used in commercial vineyard sampling sites were chosen by vineyard managers, crop consultants, and extension agents based on mildew management concerns. Fungal tissue was collected from colonies on leaves, shoots, or inflorescence or fruit clusters from May through October and shipped back to our research team for processing. *E. necator* DNA was extracted from each sample using a rapid Chelex DNA extraction process and analyzed for the presence of the G143A mutation.

In addition, a new technique (Figure 3) has been introduced utilizing cotton swabs to collect spores from worker gloves after performing standard vine maintenance or monitoring tasks (e.g. shoot thinning and tucking, catch-wire movement, leaf removal, crop thinning, tissue nutrient sampling , crop estimates, or pest and disease scouting). During these activities, it is likely that fungal spores are deposited on worker gloves or hands and that swabbing their hands/gloves would be a more efficient way to detect the presence of GPM or fungicide resistance than visual scouting with isolate collection or even the ToughSpot method.

Figure 3. Glove swab sampling technique: A – After manipulating the canopy and B – filling out the label, C – push the swab through its protective cover to D – expose the cotton swab and E – rub over gloves or hands. Replace into cover.

Glove samples were collected biweekly by researchers from 12 commercial vineyard blocks across Oregon's Willamette Valley. Each vineyard block was divided into three approximately equal-sized strata with a randomly selected row chosen for each stratum (e.g. Figure 4). For each row, thirty leaves from ten vines spaced at regular intervals along the row were visually inspected for mildew incidence and severity while simulating canopy maintenance tasks. The disease incidence was recorded and if mildew was identified, a direct colony swab sample was collected by rubbing the swab on the leaf (leaf swab). Cotton swabs were used to collect direct colony samples as they were determined to have 100% agreement with the results from the ToughSpot spore collections. An additional swab was used to collect any spores that may be on the gloves of the surveyor at the end of the row (Figure 2). The glove swab sample collection technique was compared to the aggregated results of the leaf swab samples using the G143A qPCR assay for QoI resistance. In addition to the sampling conducted by researchers, the participating commercial vineyard managers were given a protocol and supplies to collect samples from their field crews. Row numbers and collection dates were used to pair the swab sample results of the grower with the nearest researcher-collected glove (Figure 4).

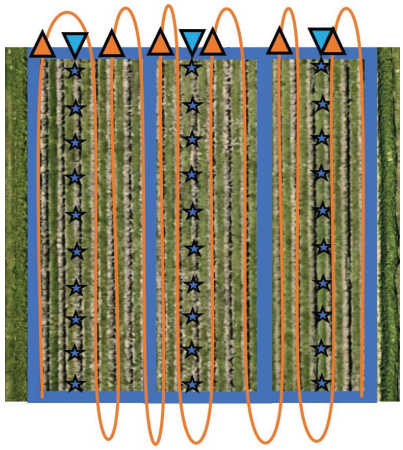

Figure 4. Sampling strategy comparing for rapid sampling methods. Blue stars indicate the researcher's leaf swabs; blue triangles, the glove swabs. Orange line indicates the path of the commercial workers; orange triangles, the glove sampling locations.

Results

Over 1400 ToughSpot samples from 273 commercial vineyards across North America were received and analyzed in 2018 for resistance to QoIs using the G143A qPCR assay (Figure 2). These samples were primarily from vineyards where the growers were concerned about resistance status in their field and displayed high frequencies of the G143A mutation. However, there was a high proportion of samples with no detectable *E. necator* DNA in early season samples, indicating that collectors

were often using visual clues that are not consistently associated with *E. necator* to misidentify infection.

To confirm the qPCR results and examine for the occurrence of the F129L and G137R mutations that have been associated with QoI resistance in other organisms (Brent and Hollomon 2007), a 966 bp fragment of the *cytb* gene was amplified and sequenced from 120 randomly selected samples collected in 2018. This sequencing indicates that the G143A mutation is still likely the only mutation conferring QoI resistance in *E. necator*.

When all leaf swab samples collected (n=572) from a vineyard row were aggregated into 326 row transects and paired to the results from the glove swab (n=421) from that row, qPCR results agreed on *E. necator* presence 66% of the time (Table 1). Only 3% of the samples had visual detection with no glove detection. For some of those sample pairs (31%), the glove swab detected mildew, but none was observed on the leaves examined. These results indicate that glove sampling may have greater ability to detect mildew in the canopy than visual assessments.

Table 1. Comparison glove swab and visual assessment for detection of *E. necator* in a row.

	Glove Detect	No Glove Detect
Visual Detect	92	9
No Visual Detect	102	123
Fisher's Exact Test, p-value: <0.001		

When *E. necator* was detected via both leaf and glove swabs, the samples agreed 98% of the time for the presence of the resistance genotype at the row level (Figure 5).

Figure 5. Paired row samples response comparisons, n=326; Red = Resistant/MT only; Yellow = Mixed/MT and WT; Green = Susceptible/WT only; Gray = Non-detection.

Additional experiments were conducted to test whether gloves needed to be changed with each row or could be rinsed with water between rows for sampling consistency. These experiments indicated that rinsing gloves with water was sufficient for removing the risk of cross-contamination from one sampling area to another, and that the expense of changing gloves was not needed.

A total of 574 commercial swab samples were collected, primarily earlier in the season (dates ranging 23 May – 18 Jul 2018) which had 78% agreement to samples collected by researchers when paired to the glove swabs that most closely matched the collection date and location (Figure 6).

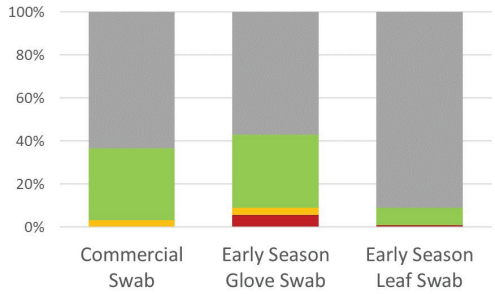

Figure 6. Commercial glove swabs and scout collected glove and aggregated leaf swabs from the samples collected from May-July. Red = Resistant/MT only; Yellow = Mixed/MT and WT; Green = Susceptible/WT only; Gray = Non-detection.

Discussion

Grape powdery mildew with resistance to QoI fungicides was detected at a high frequency across all grape growing regions surveyed (Figure 2). However, in many of the surveyed Oregon vineyards that ceased using QoI and DMI fungicides in 2017, QoI resistance was detected at a much lower frequency than in other regions still utilizing QoI or DMI fungicides (Rallos et al. 2014).

Swabbing gloves appears to be more sensitive at detecting the presence of *E. necator* than visual scouting in vineyards as well as being useful for collecting information on fungicide resistance. This could be because gloves have an increased chance of encountering *E. necator* spores while handling the canopy of each vine in the row when compared to visually examining leaves from a limited subset of the vines within a row. When conducting the systematic sampling strategy, GPM was often observed on vines which were outside of the sampling parameters (i.e. disease spotted on the return journey through the

row after completing row sampling). Other advantages of glove swabbing are that it is faster, generates less waste, and requires less dexterity to use in comparison with the ToughSpot kits. Overall, these results indicate that glove swabbing may be a viable option to monitor QoI fungicide resistance in a vineyard at a fraction of the costs and labor requirements as compared to traditional resistance monitoring. It may also provide a more accurate and rapid assessment of disease presence than traditional scouting.

Moving into the 2019 season, we hope to investigate the glove swab congruence with aerial spore sampling and whether an attachment on a tractor will be a viable way to collect conidia. Additionally, these sampling techniques for GPM can be used to test for resistance to other fungicide groups as additional molecular techniques become available. Presently, we are developing techniques for FRAC Groups 3, 7, and 13. Expanding the available methods to monitor fungicide resistance provides a direct benefit to growers in that they can monitor resistance in the field and use that information to design more effective spray programs. However, it also opens the opportunity for researchers to better investigate the emergence and persistence of resistance on a much larger scale than was previously possible.

Acknowledgements

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Literature cited

Brent KJ, Hollomon DW. 2007. Fungicide resistance in crop pathogens: How can it be managed? FRAC Monograph I. CropLife International, Brussels.

Cadle-Davidson L, Chicoine DR, Consolie NH. 2011. Variation Within and Among *Vitis* spp. for Foliar Resistance to the Powdery Mildew Pathogen *Erysiphe necator*. Plant Dis 95: 202-211.

Falacy JS, Grove GG, Mahaffee WF, Galloway H, Glawe DA, Larsen RC, Vandemark GJ. 2007. Detection of Erysiphe necator in Air Samples Using the Polymerase Chain Reaction and Species-Specific Primers. Phytopathology 97:1290–1297.

Gadoury DM, Cadle-Davidson L, Wilcox WF, Dry IB, Seem RC, Milgroom MG. 2012. Grapevine powdery mildew (Erysiphe necator): A fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Mol Plant Pathol 13:1–16.

Miles LA, Miles TD, Kirk WW, Schilder AMC. 2012. Strobilurin (QoI) resistance in populations of *Erysiphe necator* on grapes in Michigan. Plant Dis 96:1621–1628.

Qiu W, Feechan A, Dry I. 2015. Current understanding of grapevine defense mechanisms against the biotrophic fungus (*Erysiphe necator*), the causal agent of powdery mildew disease. Horticulture Research 2:15020.

Rallos L, Johnson NG, Schmale III DG, Prussin II AJ, Baudoin AB. 2014. Fitness of *Erysiphe necator* with G143A-based resistance to quinone outside inhibitors. Plant Dis 98:1494-1502.

Thiessen LD, Neill TM, Mahaffee WF. 2017. Timing Fungicide Application Intervals Based on Airborne *Erysiphe necator* Concentrations. Plant Dis 101:1246–1252.

Thiessen LD, Neill TM, Mahaffee WF. 2019. Formation of *Erysiphe necator* Chasmothecia in the Pacific Northwest United States. Plant Dis: 103(5): 890-896.

Wong FP and Wilcox WF. 2002. Sensitivity to azoxystrobin among isolates of *Uncinula necator*: Baseline distribution and relationship to myclobutanil sensitivity. Plant Dis 86:394–404.

Research publications

Results of research 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

Choudhury RA, Mahafffee WM, McRoberts N, Gubler WD. 2018. Modeling Uncertainty in Grapevine Powdery Mildew Epidemiology Using Fuzzy Logic. bioRxiv 264662.

Thiessen LD, Neill TM, Mahaffee WM. 2018. Assessment of *Erysiphe necator* Ascospore Release Models for Use in the Mediterranean Climate of Western Oregon. Plant Dis 102(8): 1500-1508.

Thiessen LD, Neill TM, Mahaffee WF. 2019. Formation of *Erysiphe necator* Chasmothecia in the Pacific Northwest United States. Plant Dis 103.

Thompson BD, Dahan J, Lee J, Martin RR, Karasev AV. 2019. A novel genetic variant of *Grapevine leafroll-associated virus-3* (GLRaV-3) from Idaho grapevines. Plant Dis 103:509-518.

Enology

Wade ME, Osborne JP, Edwards CG. 2019. Influence of must supplementation on growth of *Pediococcus* spp. after alcoholic fermentation. S AFR J Enol Vitic 40.

Wade ME, Strickland MT, Osborne JP, Edwards CG. 2019. Role of *Pediococcus* in winemaking. Aust J Grape and Wine R 25:7-24.

Extension publications

Olen B and Skinkis P. 2018. Vineyard Economics: Establishing and Producing Pinot Noir Wine Grapes in the Willamette Valley, Oregon. Oregon State University Oregon Agricultural Enterprise Budgets. AEB 0060.

Skinkis P, Pscheidt J, Moretti M, Walton V, KC A, and Kaiser C. 2019. Pest Management Guide for Wine Grapes in Oregon. Oregon State University Extension Publishing. EM 8413.

Skinkis P, Osborne J, Levin A, Kaiser C, Renquist S, and Walton V. 2019. Wine Grape Crop Production. OSU Extension Website.

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