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UNDERSTANDING SYMPTOMOLOGY AND PHYSIOLOGICAL EFFECTS OF RED BLOTCH DISEASE IN VINEYARDS IN OREGON'S WILLAMETTE VALLEY

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INTRODUCTION

Grapevine Red Blotch Disease has recently become a major concern for winegrape producers in Oregon and other areas of the US. The causal agent of the disease, Grapevine Red Blotch associated Virus (GRBaV), was first identified by researchers in California and New York (Al Rwahnih et al. 2013, Krenz et al. 2014). The disease has been at the forefront of industry concern during a time of significant industry expansion (vineyard planting) since spread has primarily been through infected nursery stock (NCPN 2017).

Anecdotal evidence from industry indicate that fruit stops ripening in the most severe cases. Studies indicate that sugar levels can lag by 1 to 2.7 °Brix (Shudarshana et al. 2015), and that fruit lack normal ripening as a result of altered secondary metabolite production that is important for wine quality (Blanco-Ulate et al. 2017). The lack of fruit ripening is a major concern for premium winegrape producers in cool climate regions such as the Willamette Valley, where ripening is a challenge in typical years due to the limited season length and heat units.

There is significant research underway to understand the virus biology and to identify insect vectors of the virus. While researchers in virology and entomology have made great strides in a matter of a few years to understand the virus-insect complex (Bahder et al. 2016), there is little definitive evidence of the impacts of the virus on vine physiology, and few research projects are focused on understanding the growth effects on grapevines.

As we seek to provide management options for growers, we need information about how the virus may be influencing vine growth and fruit ripening. We have observations from GRBaV-infected vineyard in Oregon that range from having little to no impact while others are claiming that their vineyards are no longer economically viable. The best advice to date is to remove vines that are infected and replant with "clean" plant material, but the cost of removal and replacement may not be economically feasible (Ricketts et al. 2017).

OBJECTIVES

1. Determine vine growth and physiology effects related to Red Blotch Disease in vineyards in Oregon's Willamette Valley.
2. Determine the effects of Red Blotch Disease on fruit ripening for vineyards in Oregon's Willamette Valley.

ACTIVITIES AND SUMMARY BY OBJECTIVE

Objective 1. Determine vine growth and physiology effects related to Red Blotch Disease in vineyards in Oregon's Willamette Valley

Two Pinot noir vineyards were used for research during summer 2018 for collecting symptom and vine physiological response data based on Red Blotch Disease status. We had originally planned to work with Pinot noir and Chardonnay vineyards for the two sites, but we were not able to find a Chardonnay site with known Red Blotch Disease that was causing concerns for vineyards/wineries in the region. Due to the economic impact of Pinot noir on the Oregon wine industry, we decided to use two Pinot noir vineyards to evaluate how the disease is affecting vines under two different vineyard conditions and across two AVAs. Vineyard 1 is located in the Eola-

Amity Hills AVA near Amity, Oregon and is planted (in 2007) to Pinot noir clone 828 grafted to Riparia Gloire rootstock, and it is irrigated. Vineyard 2 is located in the Dundee Hills AVA near Lafayette, OR and is planted (in 2002) to Pinot noir clone 777 grafted to 101-14 and is dry farmed (no irrigation). We used these vineyards because of the prior knowledge of Red Blotch Disease being present with symptom mapping data from the collaborating vineyard managers. In each vineyard, we were able to find a mix of virus positive and negative vines within a confined section of a vineyard block by which to evaluate vine physiological responses without having drastic soil and environmental conditions. Data collection began earlier for Vineyard 1 in 2018 since we knew virus status based on PCR testing conducted in 2017. Vineyard 2 was a new vineyard site for 2018, and virus detection results lagged due to best timing of virus testing being later in the growing season (August sampling, results available 28 Sept 2018, just before harvest). Vineyard 2 data were collected based on symptoms only in July and August until the virus testing results were available. A total of 20 vines were used for all vine growth measures in Vineyard 1, including 10 GRBV+ and 10 GRBV-, and we further classified the vines based on symptom expression (asymptomatic vs. symptomatic). For Vineyard 2, 24 vines were initially selected based on symptoms to focus on late season vine physiological measures before testing came back. However, PCR testing came back with 18 vines being GRBV+ and 6 vines being GRBV-. We retested vines in Vineyard 2 with dormant tissue samples at pruning in December 2018 to double-check the virus status for the 2019 season. If possible, we will increase our population size in Vineyard 2 to be more balanced amongst GRBV+ and GRBV- vines. Our vine numbers per grouping (virus + and –) were limited to the plant population available and the time it look for us to gather data for some of the physiological responses such as photoassimilation and stomatal conductance using a LICOR 6400 XRT in a timely fashion to ensure sound data.

Vine growth measures included vine leaf area, fruit yield, and dormant pruning weights. Leaf area was measured at véraison on two shoots per vine using a non-destructive field sampling method (Navarrete 2015). Research suggests that leaf chlorophyll begins to decline before visual symptoms are observed, so we monitored leaf chlorophyll with a SPAD meter (SPAD-502, Minolta) using leaves from three zones within the canopy (basal, mid and upper canopy) on a biweekly basis, beginning in July 2018. A pressure chamber was used for determining midday leaf water potential on several dates in Vineyard 1 during July and August. To determine the impacts of the virus on photoassimilation and stomatal conductance, an infrared gas analyzer (LiCor 6400) was used on fully exposed leaves within vine canopies on clear, cloudless days, during late summer (July-Sept). Vine tissue samples (leaf and petiole) were collected at veraison in Vineyard 1 and analyzed for macro- and micronutrients by Fruit Growers' Lab. We did not collect samples in 2018 for Vineyard 2 due to the lagging classification of the vine virus status. However, we will be able to evaluate nutrient status in both vineyards in 2019. Symptoms of GRBV was monitored throughout late summer and continuing biweekly or more frequently as we conduct growth and physiology measures. We documented canopy symptoms, fruit development symptoms, and timing of leaf fall post-harvest. Vine yield was quantified at harvest (whole vine yield weights and cluster counts per vine). Pruning weights were measured during dormancy to quantify impacts on vine vigor and determine the yield to pruning weight ratio.

Results – Vineyard 1. Leaf SPAD data (an indicator of chlorophyll) was monitored in Vineyard 1 from 17 July 2018 through 22 Aug 2018 and first began to show lower SPAD in GRBV+ compared to GRBV- vines by 7 Aug 2018 (berry touch stage) in basal leaves only and was consistent through the following sample date (22 Aug 2018). Leaves in the mid- to upper- canopy did not differ in SPAD readings, indicating a similar level of leaf greenness throughout the summer. In general, SPAD readings were high, averaging ~41, and the minimum value reported (of basal leaves) was 26. The vines were vigorous and healthy with sufficient canopy greenness throughout the summer. Vine leaf blade nitrogen was high (2.4-2.5%N) for both GRBV+ and GRBV- vines, and there were no differences by virus status. Leaf blade potassium differed by symptom status but not virus status, with asymptomatic vines having higher K than symptomatic vines at 0.99 and 0.79 % K, respectively. There were no other nutrient differences for macro- or micronutrients for leaf blades analyzed at veraison.

The first virus-associated symptoms in Vineyard 1 were observed in leaves at veraison (late Aug 2018), starting with interveinal reddening of the most basal leaves. There was little to no leaf chlorosis during the pre-veraison or post-veraison period. A slight chlorosis of leaves was visible by harvest (28 Sept 2018). By harvest, the symptoms were visible primarily in basal leaves with some occasional mid- and upper-canopy leaves having interveinal

reddening (Figure 1). Post-harvest observations revealed the majority of GRBV+ vines had delayed leaf yellowing and senescence compared to GRBV- vines in late October and early November.

Leaf photoassimilation and stomatal conductance was measured on 20 individual vines on seven dates from 5 Jul 2018 to 6 Sept 2018 to detect any potential differences based on virus or symptom status. Leaves in two zones were measured on each vine, including basal leaves and mid-upper canopy leaves. Photoassimiilation and stomatal conductance gradually declined as the season advanced, as expected with increasing soil moisture deficit and vine water stress. There was rarely a difference in photoassimilation or stomatal conductance of the mid-upper canopy leaves. However, vines without virus symptoms had higher basal leaf photoassimlation and stomatal conductance than those that were asymptomatic for two of the seven dates that this was measured. There was no difference in photoassimilation or stomatal conductance based on virus status for any of the dates measured, suggesting that vines differentially express virus symptoms and the symptoms may influence physiology more than the virus status alone.

Leaf and stem water potential were also measured on vines during three dates in August (pre-veraison and at veraison). There were no differences in leaf or stem water potential based on virus or symptom status. This is an irrigated vineyard, and drip irrigation was applied judiciously only when vines experienced stress late season. Across the three dates measured, mean leaf water potential was -0.74 MPa, -1.3 MPa, and -0.96 MPa on 1 Aug, 8 Aug, and 20 Aug, respectively.

There were no differences between vine virus status or symptom status for vine growth as measured by vine leaf area, yield, or dormant pruning weight. Vines were vigorous with high cane weights (>100 g) and low yield to pruning weight ratios from 2.2 to 2.9.

Figure 1. Vineyard 1 vines at harvest 28 Sept 2018. Mainly basal leaves had interveinal reddening by harvest and a few mid canopy leaves with this symptom. Canopies were healthy and green, another indicator of this site's vigor level.

Results – Vineyard 2. Data was collected for SPAD on three dates in late summer 2018, including 15 Aug, 28 Aug, and 6 Sept. There was lower SPAD readings (lower chlorophyll) in basal leaves of GRBV+ vines on 28 Aug and 6 Sept 2018. However, there were no differences in SPAD readings in the mid-upper canopy leaves for any

date. This accurately reflects the visual symptom expression at that stage since only the basal were showing the red coloration symptom, and mid-upper leaves had not yet shown symptoms. In general, the SPAD readings were lower than Vineyard 1, and canopies were visibly less vigorous (low to moderate vine vigor by comparison to Vineyard 1).

Photoassimilation and stomatal conductance data were collected on the same three dates from veraison to harvest as the SPAD data in the mid-upper and basal leaves within the canopy. There was lower photoassimilation and stomatal conductance in the basal leaves of GRBV+ vines and vines showing symptoms for the latest sample date 6 Sept 2018. There were no differences in photoassimilation and stomatal conductance of any mid-upper canopy leaves on any of the three late season dates measured. These data match with the leaf chlorophyll data (SPAD readings). The photoassimilation and stomatal conductance rates decreased for all vines as the season advanced, as expected. However, photoassimlation rates began to drop to a low level while stomatal conductance was high enough to not be considered in a zone of water stress (>130 mmol $H_20/m^2/s$).

The first virus-associated symptoms in Vineyard 2 were observed in leaves before veraison (early Aug 2018), starting with interveinal reddening of the most basal leaves. There no leaf chlorosis during the pre-veraison or post-veraison period. However there was chlorosis of leaves was visible by harvest (1 Oct 2018, Figure 2). By harvest, the symptoms were visible primarily in basal leaves with some occasional mid- and upper-canopy leaves having interveinal reddening. Since this vineyard block had more abiotic stress prior to harvest, leaf fall occurred earlier than in Vineyard 1, and differences in leaf abscission was not as apparent post-harvest

There were no differences by virus status for whole vine yield or yield components (cluster count, cluster weight, or berry weight) at harvest. Vine pruning weights were indicative of moderate vigor vines (~40 g canes, and yield to pruning weight ratios of 5.1-5.9).

Figure 2. Vineyard 2 vines a harvest (1 Oct 2018). Variability of GRBV symptoms are visible amongst the vines. Virus negative vines had green cabnopies without significant chlorosis or red leaves. Infected vines had variable symptoms within the vine, and not all leaves were red or chlorotic.

Objective 2. Determine the effects of Red Blotch Disease on fruit ripening for vineyards in Oregon's Willamette Valley

To determine the effect of the virus on berry ripening, we monitored the progression of véraison between healthy and infected vines at both vineyards. We initially intended to sample vines for fruit ripening up to harvest, but we were unable to do so given the individual vine experimental units, as we wanted to obtain unamended yield weights at harvest. Therefore, we waited until the block was to be harvested commercially and came in 1-3 days earlier to obtain cluster counts, yield weights, and a collect cluster samples. At harvest, we collected 2, 5-cluster samples from each vine. One of the samples was processed and analyzed for the following: cluster weight, berry count, and berry size and then pressed to juice for analysis of total soluble solids (TSS, °Brix), pH and titratable acidity. Another 5-cluster sample of Pinot noir fruit from each vine was stored at -80°C until analysis for total anthocyanin using the pH-differential method (Lee et al. 2005), total phenolics using the Folin-Ciocalteu method (Waterhouse, 2002), and total tannins using the methyl cellulose precipitation method (Sarneckis et al., 2006).

Vineyard 1 had no differences in the start or progression of berry coloration through véraison based on virus or symptom status. By harvest, there were no differences in total soluble solids based on virus status or symptom, but GRBV+ vines produced fruit with higher pH and lower titratable acidity than GRBV- vines at harvest. Furthermore, the fruit from GRBV+ and GRBV- vines did not differ in total phenolics concentration, including total anthocyanins, total phenolics and total tannins. These vines had sufficient canopy growth, vine nutrient status, and leaf greenness and a lack of differences in vine water status, leaf photoassimilation and stomatal conductance (noted in Objective 1 results), so these vines likely had sufficient capacity to ripen fruit and were not adversely affected by GRBV.

Vineyard 2 did not have any apparent differences in the start or progression of berry coloration during véraison. By harvest, fruit from GRBV+ vines was 2.1°Brix lower than fruit from GRBV- vines (23.7 and 25.8 Brix, respectively). However, there were no differences in pH (3.21) and titratable acidity (6.7). Fruit from GRBV+ vines had 14% lower total anthocyanins but 6% higher total phenolics than GRBV- fruit. Fruit from vines that were asymptomatic had 28% higher total phenolics than those with symptoms. However, there were no differences in total tannin concentration by virus or symptom status. The vines in Vineyard 2 had less green canopies by harvest and lower vine vigor than Vineyard 1, and these factors may account for difference observed at this site.

PUBLICATIONS & PRESENTATIONS

Results have been presented at industry events, including the Oregon State University Vineyard Red Blotch Disease Workshop on November 29, 2018 in Salem, OR and the Oregon Wine Symposium session on Clean Plant Materials and Red Blotch Disease on February 12, 2019 in Portland, OR. The results were shared with academic peers/industry during an oral presentation and poster session at the CDFA Pierce's Disease/GWSS and Red Blotch Disease Symposium on December 19, 2018 in San Diego, CA. This work will also be presented at a poster session at the Oregon Wine Research Institute Grape Day on April 3, 2019 and the American Society for Enology and Viticulture national conference in June 2019 in Napa, CA. No peer-refereed publications have been submitted yet. However, we will develop a manuscript for publication in a peer-refereed journal at the end of the project period.

RELEVANCE STATEMENT

This research has helped us determine the impact of the virus in two vineyards, one that is high vigor (irrigated) and one that is moderate vigor (dry-farmed). We documented limited visual symptoms and no impact on vine growth and fruit yield at harvest. Vine water status and photoassimilation was not reduced based on virus status. Given that there were few, if any, differences in nutrient status, suggests that a specific nutrient fertilization program may not ameliorate impacts of the virus. There was lower fruit Brix at harvest in Vineyard 2 where the vines had lower vigor as compared with Vineyard 1, which had high vigor and no differences in fruit ripeness, color or phenolics. With the limited differences in fruit composition observed from vineyard 1, we believe management of vine health may be the best management practice to avoid reduction in fruit quality. The second year of this work is needed to clarify consistency of symptom expression and physiology across years and to

determine whether greater environmental stress does in fact affect ability of the vine to reach optimized ripeness/quality.

LAYPERSON SUMMARY

Grapevine Red Blotch Disease is a newly identified virus of grapevines that is causing substantial concern for commercial grape producers, as it is thought to reduce fruit and wine quality. Many grape producers fear that infected vineyards will require removal and replacement, which comes at a substantial cost and may not be economically feasible. This research is being conducted to better understand how the virus affects vine growth and fruit composition. This is an important first step towards understanding how to manage the virus if possible. To date we have found no impact on vine health or productivity and limited or no impact on fruit composition at harvest under our cool climate conditions of Oregon's Willamette Valley. However, the symptoms appear more severe in the vineyard with smaller canopies and lower vigor, which are likely due to more abiotic stresses (soil, water, and nutrient) and may suggest maintaining vine health as a means to ameliorate impacts of the virus. The second year of this trial will help determine consistency of vine responses to the virus that will suggest potential vineyard management practices to be studied in the future.

STATUS OF FUNDS

Funds are being used to support a portion (0.25 FTE) of a faculty research assistant in the PI's lab. Funds have also been used for lab transportation to and from the research sites (multiple trips per week throughout summer through harvest), field supplies, laboratory supplies for fruit chemical assays, and repairs/maintenance/calibration of laboratory equipment used in assays/field studies.

SUMMARY AND STATUS OF ITELLECTUAL PROPERTY RIGHTS

There are no intellectual property rights concerns for this project.

LITERATURE CITED

Al Rwahnih M., Dave A., Anderson M., Rowhani A., Uyemoto JK., and Sudarshana MR. 2013. Association of a DNA virus with grapevines affected by red blotch disease in California. *Phytopathology* 103:1069-1076.

Bahder BW, Zalom FG, Jayanth M, and Sudarshana MR. 2016. Phylogeny of Geminivirus Coat Protein Sequences and Digital PCR Aid in Identifying *Spissistilus Festinus* as a Vector of Grapevine Red Blotch-Associated Virus. *Phytopathology* 106: 1223–30.

Blanco-Ulate B, Hopfer H, Figueroa-Balderas R, Ye Z, Rivero RM, Albacete A, Perez-Alfocea F, et al. 2017. Red Blotch Disease Alters Grape Berry Development and Metabolism by Interfering with the Transcriptional and Hormonal Regulation of Ripening. *J. Exp. Bot.*, 14.

Krenz B, Thompson JR, McLane HL, Fuchs M, and Perry KL. 2014. Grapevine red blotch-associated virus Is Widespread in the United States. *Phytopathology* 104: 1232-1240.

Lee J, Durst RW, and Wrolstad RE. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. AOAC Intl*. 88:1269-1278.

NCPN- Fact Sheet: Grapevine Red Blotch Disease. National Clean Plant Network.

Ricketts KD, Gómez MI, Fuchs MF, Martinson TE, Smith RJ, Cooper ML, Moyer MM, and Wise A. 2017. Mitigating the Economic Impact of Grapevine Red Blotch: Optimizing Disease Management Strategies in U.S. Vineyards. *Am. J. Enol. Vitic.* 68: 127–35.

Sarneckis CJ, Dambergs RG, Jones P, Mercurio M, Herderich MJ, and Smith PA. 2006. Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimized tool for grape and wine analysis. *Austral. J. Grape Wine Res*. 12:39-49. http://onlinelibrary.wiley.com/doi/10.1111/j.1755-0238.2006.tb00042.x/full

Sudarshana MR, Perry KL, and Fuchs MF. 2015. Grapevine red blotch-associated virus, an emerging threat to the grapevine industry. *Phytopathology* 105:1026-1032.

Waterhouse AL. 2002. Polyphenolics: Determination of total phenolics, p. 463-470. In: R.E. Wrolstad (ed.). Current Protocols in Food Analytical Chemistry. Wiley, Hoboken, N.J.