Grapevine Red Blotch Research Plan

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Introduction

Grapevine red blotch virus (GRBV) has been identified in many vineyards in western Oregon and has been reported to impact juice and wine quality. Grapevine red blotch disease (GRBD) was first described in 2008 in California as a distinct disease, but likely was present long before that but confused with grapevine leafroll disease. GRBV was identified in 2012, and research on the virus, its vectors, and impact on yield and fruit and wine quality has been underway since then in California, New York, Oregon, and Washington. There is still very little known about the vectors of this virus, with a single report of transmission by treehoppers (CA) and leafhoppers (WA), and no documented vector transmission in Oregon. It takes time to understand the virology, vectors, transmission, and their complex relationships, and effects on vine physiology, and grape and wine quality.

Current management recommendations focus on the use of clean plant materials for new vineyards or for replanting sections of vineyards. For established vineyards, modifications to vineyard and wine making practices may provide additional solutions. Information on virus transmission and vector management is necessary before replanting can be recommended as a management strategy for the virus. The Oregon Wine Research Institute (OWRI) at Oregon State University (OSU) began initial work on RBST in 2012 and assembled a multi-disciplinary team (i.e. RBST) in 2017 to focus on GRBV, with the goal of producing science-based management recommendations for the wine industry. The RBST focuses currently on the virus, vector(s) identification and transmission, and the impact of GRBV on vine physiology and wine quality.

We will also establish a comprehensive red blotch website that makes information on GRBV available to industry, outlining the research efforts underway in Oregon and nationwide.

Domaine Serene (DS) has presented the RBST with a research gift of $500,000 to accelerate current GRBV research, with the possibility of an additional $500,000 in wine industry matching funds. RBST research described herein will begin in May 2018, and will extend and supplement current projects within a two year (or less time) frame. The initial effort by core RBST members is based on an initial budget of $500,000 and will inform the future course of research as more funds become available, with the goal of understanding the nature, mechanisms and impact of the GRBV, leading towards management recommendations and a possible virus vaccine.

Objectives:

1. Develop and manage online content for GRBV – Skinkis
2. Evaluate genetic diversity of GRBV in the Willamette Valley--Martin
3. Identify the vector(s) of GRBV in the Willamette Valley – Martin, Lee (Jana), and Walton
4. Map the virus in DS vineyard – Martin
5. Understand vine physiological and fruit quality impacts in Willamette Valley Pinot noir – Skinkis
6. Determine the impact of GRBV on quality of Willamette Valley Pinot noir – Enology team (Lee (Jungmin), Osborne, Qian, Tomasino)

Expected Outcomes of this Project:

1. A website with current and comprehensive GRBV information.
2. Knowledge of the genetic variability of GRBV in the Willamette Valley, which will be used in the future to improve detection and to identify RNAi targets for development of disease management vaccines.
3. Identification of insect vector(s) and transmission of GRBV in the Willamette Valley.
4. Baseline data on GRBV actual virus distribution in the vineyard to supplement existing data based on symptoms. This information will inform efforts to assess the effects of GRBD on vineyard health, fruit quality, and chemical properties of wine.
5. Knowledge of the influence of vineyard management practices on health, physiology and fruit quality of GRBV infected vines.
6. Knowledge of the impact of GRBV on volatiles, phenolics, and sensory components of wine.

Communication is a key to a successful partnership and outcomes. Leigh Bartholomew (Results Partners) and Mark Chien (OWRI) will act as research liaisons between DS and OWRI, and offer quarterly updates and an annual report to both teams, as well as facilitate research and education needs and meetings as they arise. The goal is to provide the DS production team and growers in the Willamette Valley as a whole with scientifically-based options for the management of GRBV.

This project will be conducted in the Willamette Valley with a primary focus in Yamhill County. OWRI has additional research faculty at the OSU Southern Oregon Research and Extension Center (SOREC), including a viticulturist and plant pathologist, who are actively working on

GRBV in that region, and research data will be shared between research partners for maximum knowledge and benefits, as well as other research institutions. All research derived from this partnership should be publishable. Donors and cooperator identities will be kept confidential. Additionally, several of the researchers on this team are also involved in a large national Specialty Crops Research Initiative (SCRI) proposal on GRBV and have applied for research funds from the Oregon Department of Agriculture, Oregon Wine Board, American Vineyard Foundation and other research funding organizations and agencies that will complement the work described here.

Individual Research Components

1. Develop and manage online content for GRBV– Skinkis

There is currently no comprehensive online source of information for GRBV. To ensure that industry is aware of new findings, information gathered from published research studies and current projects will be posted on a GRBV webpage. Work will be summarized based on the virus, vectors, impacts on grapevines, and potential management options. Information will be technically written in a concise yet informative manner for use by an industry audience. This work will allow the industry access to a centralized location of information from this and other projects, as it comes available.

2. Evaluate genetic diversity of GRBV in the Willamette Valley - Martin

The diversity of GRBV in vineyards throughout the Willamette Valley will be determined by nucleotide sequencing. We plan to sequence 15 to 20 samples of GRBV obtained from vineyards located throughout the Willamette Valley. GRBV DNA isolated from the petiole samples of infected vines will be amplified by PCR and sequenced, and sequences will be analyzed bioinformatically.

Expected outcomes:

- Knowledge of the prevalent GRBV isolates, their distribution in the Willamette Valley, and their relationship to isolates present throughout the US.
- The sequence data will provide baseline information needed for future studies aiming to optimize detection of GBRV and to design an anti-GRBV vaccine using RNAi.

3. Identify the vector(s) of GRBV in the Willamette Valley - Martin, Lee (Jana), and Walton

Identifying the vector of GRBV is key to developing disease management strategies in the future, and a primary objective of this research plan. GRBV is in the large and diverse Geminiviridae family, and four large groups of insects (aphids, leafhoppers, tree hoppers, and whiteflies) are known vectors of this family of viruses. To date, two vectors of GRBV have been identified: the Virginia creeper leafhopper (*Erythroneura ziczac)* and the three-cornered alfalfa hopper (*Spissistilus festinus*, a tree hopper) have been shown to transmit GRBV from infected to healthy grapevines in Washington and California, respectively. Previous work by the

research group of RBST member Vaughn Walton showed that the tree hoppers *Tortistilus albidosparsus* and *T. wickhami* are present in Willamette Valley vineyards where GRBD is spreading and carries GRBV. *Spissistilus festinus*, has also been found in this region based on collections by the Walton laboratory in Benton county and from the OSU insect collection records. To date, however, the GRBV vector(s) important in spread of GRBD in the Willamette Valley is unknown.

Due to the importance of identifying a vector, we plan a two-pronged approach for this objective. The Walton team will focus primarily on the leafhoppers and tree hoppers, groups shown to transmit GRBV in other locations. The Martin-Lee team will do a broad screen for GRBV transmission, an inclusive approach aiming to reveal vectors that may be missed in a more targeted study.

3a. Screen a broad collection of insects from the DS Two Barns vineyard for transmission of GRBV –Martin and Lee (Jana)

From the DS Two Barns vineyard, diverse insects will be collected using sweep nets, beating trays and bug vacuum, every two weeks during the growing season. Insects will be transferred to the USDA-ARS Lab (Corvallis, OR) where insects will be sorted into taxonomic groups based on visual characteristics and then each group will be caged on infected plants for 5 days to ensure they have the opportunity to acquire the virus. After 5 days, 4 healthy plants will be added to each cage and the insects will be allowed to feed for an additional 7 days. After the 7 days transmission period, the cages will be fumigated and the healthy plants moved to a screen house. This will be repeated every two weeks until harvest. All plants will be tested for GRBV at the end of the growing season and maintained for testing at the end of the 2019 growing season again, since we do not know the time required for GRBV detection by PCR following acquisition of the virus by the plant through insect transmission.

3b. Survey vineyards for potential GRBV vectors and evaluate candidate vectors for GRBV transmission - Walton

Insects will be collected from two vineyards in the Willamette Valley with a history of GRBV spread. We will survey using yellow sticky traps placed along the border (4 border traps/vineyard) and center (2 center traps/vineyard) of selected blocks. Trap collections will be conducted twice per month starting in May and continuing until November (14 sampling dates), typically the period when most of the listed insect vector species are present at higher population levels. Intense insect vector sampling will additionally be conducted using sweep-nets, D-Vac and visual inspection techniques. Here we will use a systematic sampling pattern in a minimum of 40 locations of each study vineyard. This methodology will allow spatial analysis of vectors in each of the vineyards in order to determine ecological importance. Collected insects will be taken to the laboratory for controlled rearing and transmission biology experiments. Subsamples of collected insects for each sampling location and date will be directly placed into separate vials containing 95% ethyl alcohol. These samples will be investigated under a stereomicroscope to determine species composition of the most likely vector insects. A subsample of vector insects will be processed for GRBV detection using PCR analysis.

<u>Expected Outcomes:</u>

- Identification of the primary insect vector(s) of GRBV in the Willamette Valley
- Information will be used to design follow up projects on the mechanism of transmission and vector host ecology and preferences leading towards GRBV management strategies.

4. Map the presence of GRBV in the DS vineyards and relate to GRBD symptoms – Martin

Knowledge of the distribution of GRBV in the DS vineyards is needed for the studies evaluating the impact of GRBV on grapevine physiology and wine quality (Objectives 5 and 6). To gain this knowledge, we will evaluate 400 field vines (on three rootstocks and multiple blocks) from DS vineyards during the summer and fall of 2018 for GRBV. DNA isolated from the petiole samples of vines will be amplified by PCR using primers specific to GRBV, and the presence of a PCR product will indicate the presence of GRBV. Information from this analysis will be used to annotate the existing map of the DS vineyards with the presence or absence of the virus in relation to the severity of the observed GRBD symptoms. From this map, plots for the studies of Objectives 5 and 6 can be designed.

We also plan to use these samples to pursue an explanation for our previous observations that the severity of GRBD symptoms is not strictly correlated with the presence or absence of GRBV. It is possible that GRBD is more severe when GRBV is present in mixed infections with other viruses, a phenomenon seen in other plant virus disease complexes. To pursue this possibility, we will focus on three candidate viruses we detected in symptomatic grapevines in another Oregon vineyard in 2016 and 2017. The three candidate viruses were sequenced, so we can use PCR to determine if they are present in the 400 samples of this study. By annotating the vineyard map for the presence of the three candidate viruses, as well as GRBV and GRBD symptoms (as described in the paragraph above), we can determine if GRBD symptoms are more severe in grapevines infected with more than one of the viruses.

Expected Outcomes:

- Baseline data on GRBV actual virus distribution in the vineyard to supplement existing data based on symptoms.
- This information will inform efforts to assess the effects of GRBD on vineyard health, fruit quality, and chemical properties of wine.

5. Understanding red blotch disease vine physiological and fruit quality impacts in Willamette Valley Pinot Noir – Skinkis

Understanding the physiological impacts of the virus on grapevines in the cool climate of the Willamette Valley is the first step towards developing management plans for infected vineyards. Without this information, we cannot recommend management strategies, whether it is to remove the vineyard, replant, or ameliorate the impacts of the virus. There is little research to date on the vine physiological effects of the virus, as efforts focused on the virus biology, vectors, and transmission. Evaluating grapevine symptoms and physiological-responses across multiple growing seasons will help us better understand impacts of the virus on vine growth, ripening and fruit/wine quality, particularly under varying climatic conditions from season to season.

Delayed Symptoms or Virus Spread? The appearance of red leaf symptoms across a vineyard block is frequently interpreted as virus spread, but it is unclear whether the appearance of symptoms is due to virus movement (transmission) to nearby vines or whether it is delayed expression of symptoms in already infected vines. It is hypothesized that symptoms vary across blocks in relation to soil profile differences, and vines located in areas with lower soil moisture, thinner soils, or low soil fertility, experience earlier or more severe symptoms. With time, symptoms may appear to spread when vines may be reflecting the impact of the environment (dry summer/season) or the soil type within the vineyard where soil type or properties shift rather than being a result of new and progressing infections.

Expected outcomes:

- Develop an understanding of vine physiological responses that may lead to further research and further management trials.
- Provide industry with information pertaining to vineyard management practices that may enhance vine health, fruit and wine quality.

6. Determine the impact of GRBV on quality of Willamette Valley Pinot noir--The Enology Team –Lee (Jungmin), Osborne, Qian, Tomasino

The impact of GRBV on Oregon wine quality has not been scientifically documented, although uneven ripening (decrease in sugar, color, and increase in titratable acidity) have been reported in CA-grown GRBV infected Zinfandel (Blanco-Ulate et al., 2017). Given the distinctly different viticultural challenges facing premium Pinot Noir producers in the Willamette Valley compared to other winemaking regions, there is a need to understand how GRBV impacts Oregon Pinot Noir wine. To do this, it is necessary to produce wines using Pinot Noir grapes from GRBV infected and healthy vines (confirmed by virus-testing) under carefully controlled winemaking protocols and assess wines for variables known to affect wine quality parameters. Collecting analytical information as well as conducting sensory analysis of the wines will determine the significance of changes in volatile and phenolic compounds by linking them to discernable differences in the sensory qualities of the wine.

Expected outcomes:

- Results from this study will provide the first information about the specific impact of red blotch on key Pinot noir wine components such as aroma compounds and phenolics.
- Understanding these changes will help the development of management strategies that could be used in the winery to mitigate any quality issues.

Next steps:

GRBD research will evolve as the RBST and other researchers achieve a greater understand of GRBV. The projects described herein will likely evolve to include more intensive studies of virus and vector ecology, and potential genomic solutions to managing the disease. Based on initial results and the availability of additional funds, research can be extended. For example, once the vector(s) is definitely identified, projects on mechanism of transmission and vector host ecology and preferences will be added to the project leading towards GRBV management strategies. These initial results would also help inform the possible design of an anti-GRBV

vaccine using RNAi methods currently being employed to develop grapevine leafroll virus vaccines. For this future work, other team members will be added to the project as needed. RBST faculty are seeking additional support from the Oregon Wine Board, Oregon Department of Agriculture, USDA Specialty Crops Initiative, California Department of Food and Agriculture, and other funding agencies to further empower their research efforts, along with developing powerful multi-region and disciplinary research relationships. RBST will be in close contact with DS as planning and implementation of GRBV research continues.

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