ULTRASTRUCTURAL ANALYSIS OF GRAPEVINE RED BLOTCH DISEASE

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BACKGROUND

Grapevine Red-Blotch Disease (GRBD) caused by Grapevine Red-Blotch Virus (GRBV) is a significant viral disease of wine grape varieties (*Vitis vinifera*), infecting all major grapevine growing regions on a global scale. On a physiological level GRBV disrupts photosynthetic efficiency and host metabolism by prematurely reddening leaves (red-fruited varieties), which is typically observed during the critical phase of post-véraison berry ripening (Figure 1). The red leaf color signifies a build up of sugar in the source leaves, leading to the development of protective red anthocyanin pigments. This process signifies an over accumulation of sucrose in the leaves (source) as a result of the phloem transport becoming blocked at the sieve tubes and more specifically sieve tube plate pores. This results in a berry with a reduced sugar content, aromatic compounds, and an abnormal development of grape berries (Figure 2). These GRBV leaf symptoms appear similar to grapes infected with grapevine leaf-roll disease(GLD), another significant virus in grapevines.

INTRODUCTION

Current GRBV infection dynamics within the grapevine host tissues and the potential resistance mechanisms are yet to be understood. Understanding how GRBV moves through the cellular network of various grapevine cultivars is expected to provide insight into where and when the virus replicates in the plant, how fast it spreads within the vine, the downstream consequences, and what tissues and functions are inhibited/induced/altered during the infection. Our overarching goal is to address these aspects and aid grape growers in formulating innovative management strategies to minimize its incidence and eliminate GRBV spread in vineyards.

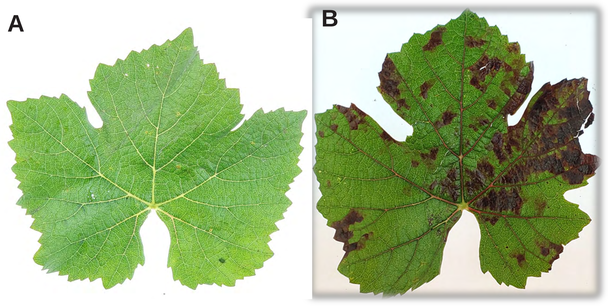

Figure1: (A) Healthy Pinot noir leaf and (B) GRBV-infected Pinot noir showing typical symptoms of GRBD

GOALS AND OBJECTIVES

Investigate GRBV's movement within the grapevine using ultrastructural analysis techniques, specifically, scanning (SEM) and transmission electron microscopy (TEM) (Figure 3). More specifically,

•Determine cellular transport routes the virus uses to disperse infection to the whole plant

•Specify the barriers and plant defense mechanisms the virus overcomes

MATERIALS AND METHODS

Field Study Sites

- Benton City, WA, Amity, OR, Medford, OR

•Varietals: Merlot, Pinot noir

•Partner with **OSU** (testing of GRBV)

•Individual Vine Analysis: 6 infected and 6 control at each site. Sampling frequency occurred at one month intervals August to October for temporal analysis of disease progression

•Tissue selection for TEM and SEM analysis: Leaf Veins: Primary & Secondary, Leaf Tissue, Petiole, and Roots

•2 clusters from each vine (12 healthy and 12 and infected clusters) were run for a basic juice berry composition analysis

•Fresh cluster weights and berry weights were sampled to monitor any disease effect on berry weight averages

RESULTS

- Visually clusters of infected vines were smaller with fewer number of berries (Figure 2)
- Individual berry weight averages were lower in infected vines throughout the season (Figure 4)
- Chloroplasts of infected vines consistently show a greater amount of starch build up (Figure 5) with TEM analysis
- Chloroplasts of infected Merlot vines become completely dismantled with grana stacks floating in the cytoplasm of the cell(Figure 6)
- Surface structure analysis with SEM, shows a severe disruption of the cellular structure in the grapevine tissues (Figure 7)

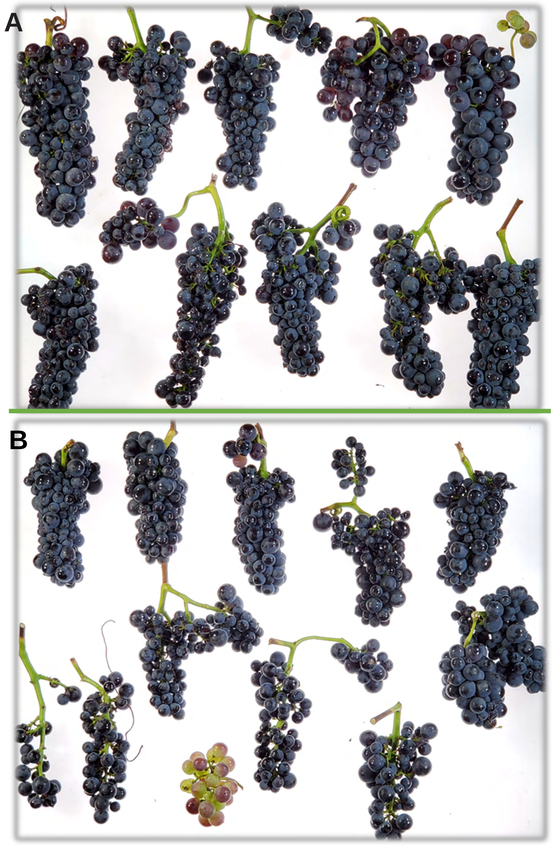

Figure 2: (A) healthy Pinot noir clusters and (B) GRBD infected (August 27, 2020: Amity-Eola Hills, Oregon).

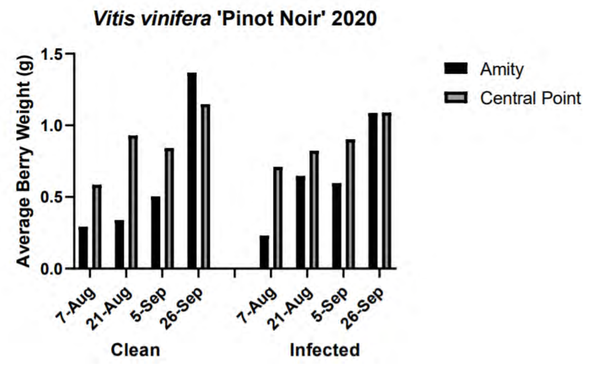

Figure 4: 2020, On average fresh berry weights are lower in the Infected vines more noticeable towards fully berry maturation .

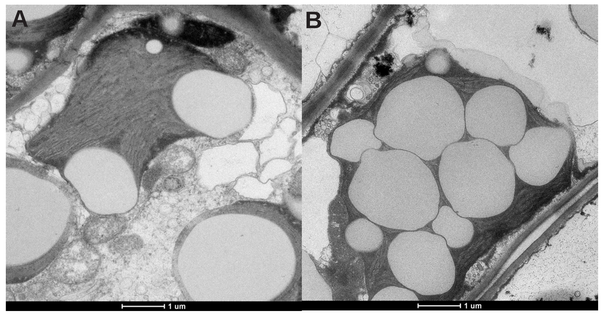

Figure 5: (A) Healthy leaf with relatively less starch accumulation and (B) Extensive build up of starch in infected leaf in Merlot grapes.

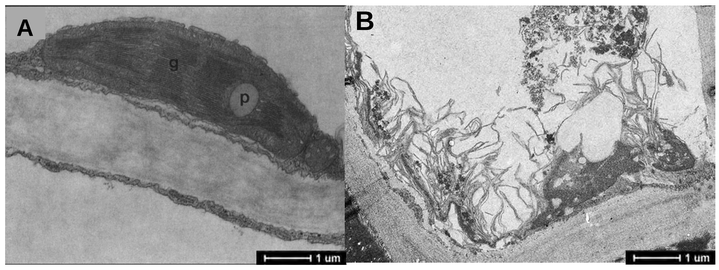

Figure 6: TEM analysis of (A) Normal granum (g) stacks of a healthy chloroplast with plastoglobuli (p) and (B) complete dismantling of the granum stack of the thylakoid discs infected Merlot vine (Oct. 27, 2020: Benton, WA).

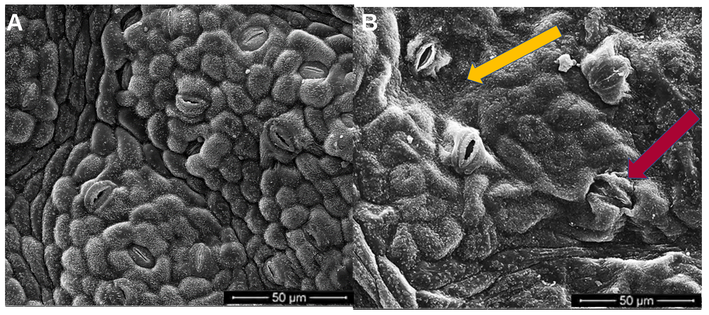

Figure 7: Surface morphology of (A) healthy Merlot leaves with normal cellular structure and (B) infected Merlot leaves showing sunken stomata (red arrow) and collapsed cells (yellow arrow)

CONCLUSIONS

- Previous studies show GRBV infected vines have a significantly lower amount of TSS (total soluble solids), anthocyanins, and seed phenolics (Copp et al., 2020)
- Symptomatic leaves consistently have higher nonstructural carbohydrates, sucrose, and starch (Poojari et al., 2013). This explains why the chloroplasts of the infected vines are storing more starch (Figure 5, left image) also indicating the phloem, which is responsible for sugar transportation, may be responding to viral infection by plugging its sieve plate pores with callose deposition
- A study on Geminivirus, radish leaf curl disease (RaLCB), revealed localization of the DNA virus-encoded protein in the chloroplast ultrastructure, which lead to structural deformation of the organelle and disruption of photosynthetic machinery (Bhattacharyya et al., 2015)
- Prior research shows GRBV + vines had a decrease in leaf gas exchange post-véraison (Levin et al., 2020) which may contribute to our surface structure analysis (Figure 6) showing an alteration in stomata structure

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