

COMPARATIVE DIAGNOSTIC METHODS FOR GRAPEVINE RED BLOTCH VIRUS (GRBV)

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INTRODUCTION

Grapevine red blotch disease (GRBD) is a newly identified disease of grapevines caused by grapevine red blotch virus (GRBV). The virus is a single stranded DNA virus within the family Geminiviridae. Since its discovery in 2008 in California, several major grape-growing areas in North America have been severely impacted by the disease. The economic losses associated with the disease is reduction in fruit quality and delayed ripening. The disease alters fruit juice chemistry, and reduces the total soluble solids (TSS) up to 5° Brix.

Most grape cultivars are susceptible to the disease with differential symptom expressions on white-fruited and red-fruited varieties. Symptoms are more apparent in red-fruited varieties where the foliar tissues consist of red blotches early in the season which expand and coalesce across leaf blades as the season progress. Diagnosing red blotch as characteristic symptoms of disease is very challenging as the vines produce similar symptoms during different biotic and abiotic stresses. A confirmation of visual symptom for the presence of virus is necessary. Currently several DNA-based assays are available for accurate disease diagnosis, including loop-mediated isothermal amplification (LAMP). The LAMP assay is purportedly more sensitive, quick, and affordable than PCR-based diagnosis. However, with limited field testing, it is difficult to fully rely on the test results to determine full impact on vineyard. In 2020, we conducted an experiment to compare the accuracy of three GRBV detection methods in samples collected from a commercial vineyard in southern Oregon.

MATERIALS AND METHODS

- A commercial vineyard in southern Oregon with history of GRBD was selected for this study. The block consisted of Pinot Noir (clone 777) on rootstock 3309C. The vines were planted in 2010 at 2.0 m x 1.5 m between the rows and vines respectively.
- Vines were naturally infected, and their GRBV status was predetermined from year-end sampling in 2019.
- Twenty vines each of GRBV positive and negative from 2019 sampling were selected for the study
- Leaf samples were collected from basal, middle, and apical shoot nodes at berry set, veraison, and harvest.
- Each sample consisted of two whole leaves (blades with attached petioles) collected from each bilateral cordon.
- Samples were rated for GRBD symptoms at the time of collection, and subsequently tested for GRBV infection using LAMP, PCR, and qPCR assays.



Figure 1: Canopy and stages at which samples were collected for GRBV testing.

RESULTS

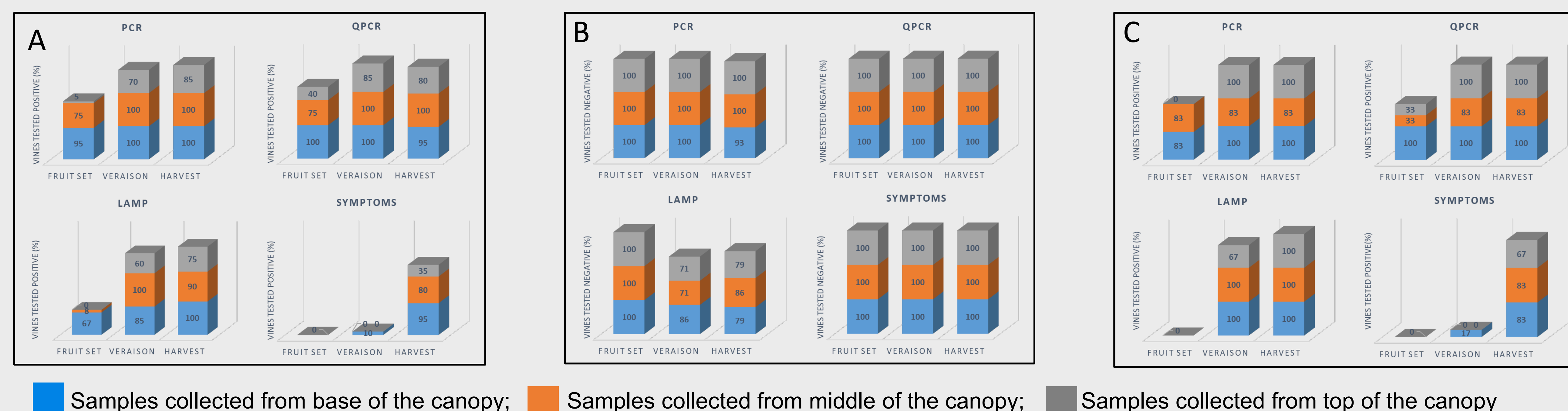


Figure 2: GRBD diagnosis of the samples that were naturally infected and tested positive in previous year (2019) sampling (A); tested negative in previous year sampling (B); and tested negative in previous year sampling but tested positive in current year (2020) sampling (C).

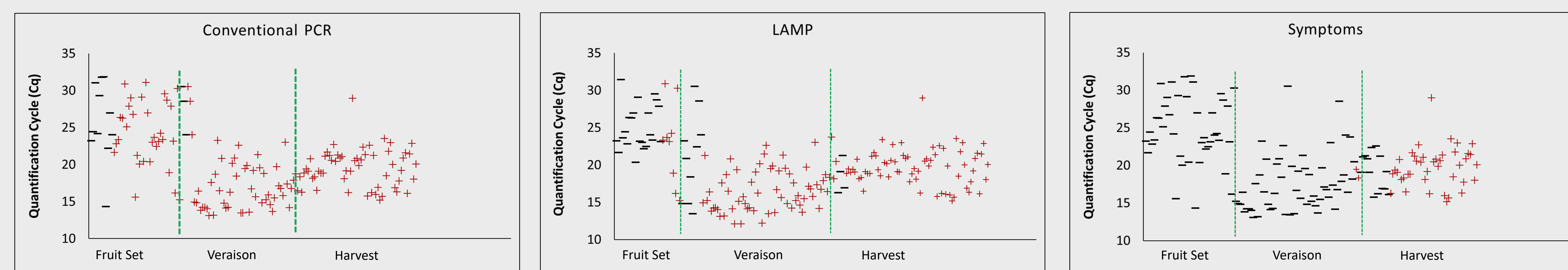


Figure 3: Virus concentration and their detectability at different stages of crop development. The quantification cycle threshold (Cq) obtained by qPCR was plotted against the virus status as diagnosed by conventional PCR, LAMP, and symptoms. '-' represents the negative status and '+' represents the positive status.

- All vines marked as positive in 2019 tested positive in 2020 in one stage or the other
- Only 14 vines out of 20 vines (70%) marked as negative in 2019 tested negative, indicating 30% increase in GRBV infection in one year within the same plot
- Six out of 20 vines (30%) were marked as new positive in 2020
- False negative ratios were significantly less at basal petiole samples at veraison and harvest for all methods. It was true for the new positive vines as well.
- All methods except LAMP detected 100% of the negative vines across all stages and canopies. LAMP detected 76% and 81% of the total negative samples at veraison and harvest respectively
- The relative concentration of GRBV at fruit set is significantly lower at middle and top of canopy. The concentration increases significantly at all canopy levels at veraison; however it decreases at harvest.
- The variability in virus concentration within all tissue type is minimal at harvest

CONCLUSION

- Older leaves and late season petiole samples increase accuracy of GRBV diagnosis.
- Virus detectability by PCR, LAMP, and symptoms expression are not directly correlated with virus concentration
- Towards harvest we maximize the possibility of selecting right tissue samples and minimize variability associated with GRBV detectability
- With right type of tissues and stage of vine development all methods accurately detects positive vines; however the sensitivity of LAMP assay to negative vines needs further validation.

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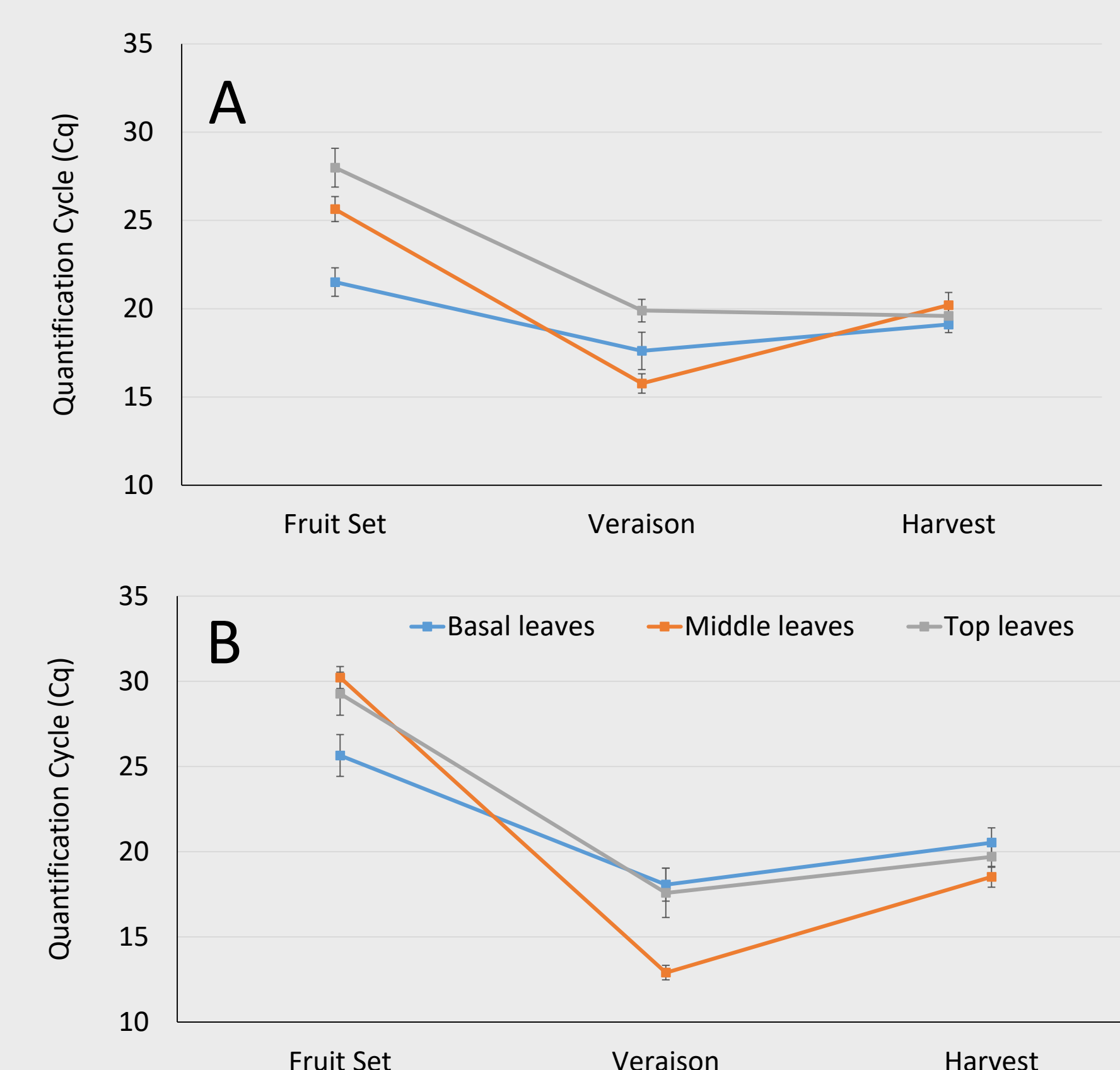


Figure 4: Relative quantification of virus concentration at different stages of vine development: A) vines that tested positive in previous year sampling, B) tested negative in previous year sampling but tested positive in current year sampling.