Grapevine Leafroll Associated Virus -3 Seasonal Titer and Effects on Pinot Noir Fruit in Oregon

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Introduction

The Willamette Valley, west of the Cascades in Oregon, is home to a growing number of vineyards. This region is best known for producing high quality Pinot noir wines. *Grapevine leafroll associated Virus -3* (GLRaV-3), genus *Ampelovirus,* family, *Closteroviridae*, which is transmitted by mealybugs and scale insects, is a new threat to this industry. If mealybugs are present, growers use pesticides to prevent them from spreading the virus.

GLRaV-3 causes reddening in leaves and uneven ripening of the fruit that leads to negative effects on phenolic composition, color development and skin elasticity in grapes as they ripen.[1,2] The impacts of GLRaV-3 on fruit, can lead to major impacts on wine quality.

Detection of GLRaV-3 by ELISA and PCR tests is more reliable later in the season (September) than early (June). If virus titer is correlated with ease of detection, the virus may not be transmitted early in the season, and then pesticide application during this 'low titer' time would be unnecessary.

Materials and Methods

- Young, middle aged and mature leaf tissue was collected weekly during the growing season, from 6 vines each of self-rooted ('Pommard' clone) and grafted (clone '114' on 'Gloire' rootstock) infected with GLRaV-3 from a commercial vineyard in the Willamette Valley,
- GLRaV-3 RNA was quantified using AgPath-ID™ One-Step RT-PCR Kit (Applied Biosystems, USA), with primers we designed for amplification and the Taqman® probe (Fig. 1). A qPCR assay for the host RNA of the NADHβ subunit, was used to monitor RNA extraction, RT, and PCR efficiency and standardize RNA concentrations.
- GLRaV-3 titers were compared to the titer obtained the first week and to healthy plants.

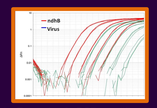

Figure 1. Example of qPCR amplification curve with 10-fold dilutions.

- 864 berries representative of four ripening transition classes were tagged (colored strings) on 3 healthy and 3 GLRaV-3 infected vines; 6 berries per class (n=4) per cluster (n=6) per plant (n=6).
- Each week from *mid-véraison* to harvest, one berry per class, per cluster, per plant was sampled, n=144.
- Samples were measured for elasticity using an adapted skinfold caliper, color using a chromameter with CIELAB parameters[3] for red grapes, and total soluble solids (brix degree) using a digital refractometer. Comparisons between healthy and infected grapes were made for a 5-week span.

Results

The Δ ΔCt values in each graph below show relative exponential increase, assuming 100% PCR efficiency, of virus titer compared to Week 1 samples. The graphs from 2010 contain some samples that did not properly amplify, which are visible in the line breaks. Virus titers in young leaf tissue from each group increased by as much $2^{(12)}$ ($2^{(\Delta\Delta Ct)}$), approximately a 4000-fold increase, during the growing season for both years. In middle aged leaf tissue the virus titer increased by $2^{(7)}$ during the growing season in both years. With the Pinot noir clone '114' grafted onto 'Gloire' the virus titer in the oldest leaves in 2010 increased by $2^{(7)}$, while in self-rooted 'Pommard' the RNA was not amplified for ndhB, suggesting problems with the RNA extraction from that tissue. In 2011, the virus titer in the oldest leaves of both cultivars increased; by $2^{(6)}$ for '114' on 'Gloire', and $2^{(4)}$ for the 'Pommard', respectively, relative to the target sample.

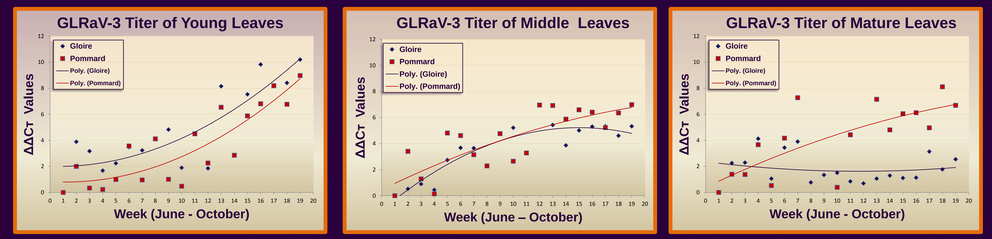

Figure 2. Year 2010 scatter plots of each sample; relative quantification of GLRaV-3 titer is measured as ΔΔCt values against weeks throughout growing season. Sampling began the first week of June and continued for 20 weeks.

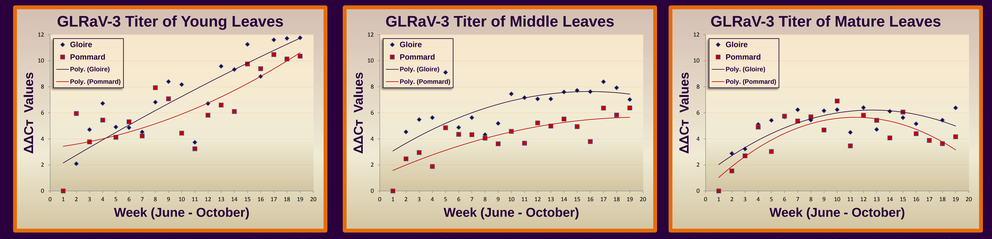

Figure 3. Year 2011 scatter plots of each sample; relative quantification of GLRaV-3 titer is measured as ΔΔCt values against weeks throughout growing season. Sampling began the first week of June and continued for 20 weeks.

Mean sugar content values in healthy berries converged around 26% by harvest in each mid-verasion class; whereas berry classes from GLRaV-3 infected plants remained highly variable. Mean elasticity measurements displayed an unusual trend suggesting errors in recording or measurement. However, the lines for healthy berry classes still converged by the last week more than infected. Mean color index results indicated that healthy mid-verasion berry classes developed more color by harvest than infected classes, which also show unevenness near the final week.

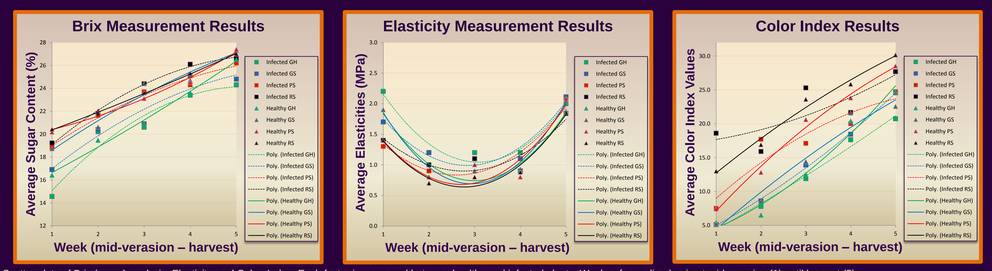

Figure 4. Scatter plots of Brix (sugar) analysis, Elasticity, and Color Index. Each factor is compared between healthy and infected plants. Weeks of sampling begin at mid-verasion (1) until harvest (5).

Conclusions

- GLRaV-3 virus titer shows a dramatic increase during the month of June, suggesting transmission by vectors could occur during most of the season. Week –to-week fluctuations once maximum titer is reached may be due to environmental conditions, such as collection on extremely warm days.
- GLRaV-3 titer in younger leaves builds throughout the growing season.
- RNA from ndhB in the 2010 'Pommard' mature collection was not thoroughly extracted during the season.
- Sugar content, elasticity and color index properties of four berry ripening classes at mid-verasion for infected plants all show variation.
- Berries of healthy plants show relative convergence among mid-verasion classes for all properties.

Figure 5. 'Pinot Noir' wine grape (*Vitis vinifera*) with grapevine leafroll virus (GLRaV-3). Copyright Melodie Putnam 2008, Oregon State University

References

[1]Lee, J. and Martin, R.R. (2009) Influence of grapevine leafroll associated viruses (GLRaV-2 and -3) on the fruit composition of Oregon Vitis vinifera L. cv. Pinot noir: Phenolics. *Food chemistry* 112: 889-896.

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[3]Carreno J, et al. (1995) Proposal of an index for the objective evaluation of the colour of red table grapes. *Food Research International* 28:373-377.

Acknowledgements

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