Maintaining healthy grapevines in registered nurseries through virus diagnostics

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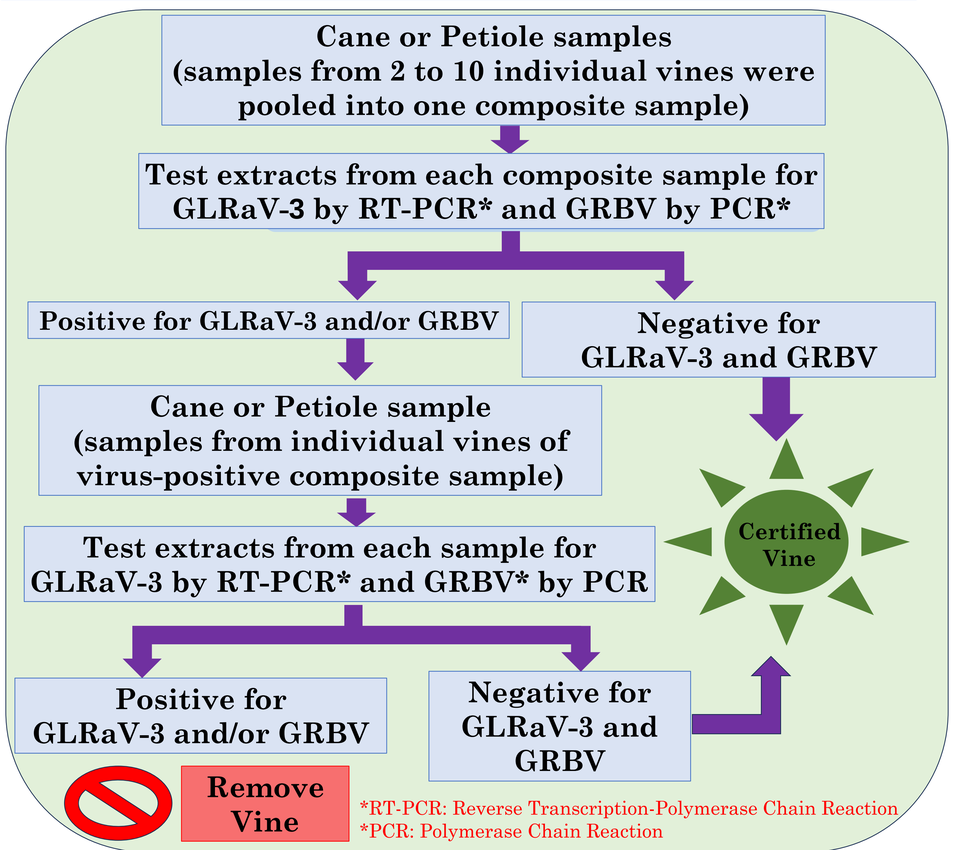
Introduction

Washington State is one of the world's premier wine grape-producing regions in the world (Fig. 1). The grape and wine industry's impact on the state's economy was estimated to be ~$9.6 billion in 2017 (https://wineamerica.org/policy/by-the-numbers/). Viral diseases are recognized as a significant constraint to the sustainability of Washington's wine industry. Among the several viral diseases, Grapevine leafroll (GLD) caused by *Grapevine leafroll-associated virus* 3 (GLRaV-3) and grapevine red blotch (GRBD) caused by *Grapevine red blotch virus* (GRBV) are considered the most economically important diseases in the state. GLD and GRBD affect vine health, cause reduced fruit yield and delayed fruit ripening and affect quality of grapes leading to economic loses to growers.

Maintaining healthy grapevines in registered nurseries is critical to provide virus-tested planting materials for growers to plant new vineyards. Towards this objective, the Grape Virology Program at Prosser IAREC has been collaborating with grapevine nurseries and Washington State Department of Agriculture's Plant Services Program to ensure vines in mother blocks of registered nurseries are tested free from economically important viruses. For this purpose, leaf and cane samples from mother vines of wine grape cultivars and root stocks were tested by molecular diagnostic techniques for the presence of GLRaV-3 and GRBV. A limited number of samples were also tested for GLRaV-1 and GLRaV-4, and the bacterium causing Pierce disease.

Fig. 1. Washington State map showing major grapevine-growing regions.
Source: *www.washingtonwine.org*

Methodology



Results

A composite sampling strategy was used for testing more vines from registered mother blocks

- Leaf (petiole) samples during the crop season and cane samples during the dormant period were collected from 2 to 10 individual vines and pooled into one composite sample.
- Extracts made from each composite sample were tested for GLRaV-3 by RT-PCR and GRBV by PCR in our laboratory at WSU-IAREC.
- Approximately 10% of composite samples were also tested by molecular diagnostic assays for GLRaV-1, GLRaV-4, and the Pierce disease bacterium (*Xylella fastidiosa* subsp. *Fastidiosa).*

Absence of GRBV and low incidence of GLRaV-3 in samples from registered mother blocks

- In 2023, cane samples collected during the dormancy period and petiole samples collected during the crop season were tested for GLRaV-3 by RT-PCR and GRBV by PCR using optimized sample extraction protocols.
- A total of 5,289 composite samples collected from 28,676 individual vines (3,205 vines from wine grape cultivars and 2,084 vines from different rootstocks) were tested for GLRaV-3 and GRBV (Table 1).
- The results showed 215 composite samples (212 from wine grape cultivars and 3 from rootstocks) positive for GLRaV-3. On the other hand, all composite samples tested negative for GRBV.
- Samples were collected from individual vines of each composite sample tested positive for GLRaV-3. Extracts were retested for the virus in RT-PCR. The results were used to identify virus-positive vine(s) in each composite sample (that was positive for GLRaV-3).
- The results were shared with corresponding nurseries and WSDA Plant Services Program to remove virus-positive vines from registered mother blocks.
- The results obtained in 2023 are consistent with results from previous years, indicating absence of GRBV and relatively low levels of GLRaV-3 in registered mother blocks (Fig. 2).

Absence of other GLRaVs and *Xylella fastidiosa* in samples from registered mother blocks

- A subset (10% of the total samples) of nursery samples were tested for other viruses. During 2023, a total of 560 composite samples from registered mother blocks were tested for GLRaV-1 and GLRaV-4. All samples tested negative for these two viruses.
- In addition to the GLRaVs, these 560 composite samples were tested for *Xylella fastidiosa* ssp *fastidiosa*, which causes the Pierce disease. All samples tested negative for this bacterium.

Evaluated and updated diagnostic primers used for the detection of GLRaV-1

- We optimized methods for the detection of GLRaV-1 using the coat protein-specific primers to amplify 186 bp fragment (Moran et al., 2023. Plants 12:876). This improved method can detect all known genetic variants of GLRaV-1. This optimized assay enhanced our ability to detect GLRaV-1 in grapevine samples.

Table 1. Summary of test results from samples received from registered mother blocks during 2023.

Source	Samples tested	GLRaV-3 positive	GRBV positive	Total vines
Registered Nursery 1	2608 (790*)	152 (117*)	0	14488
Registered Nursery1_rootstock	423 (12*)	2 (1*)	0	1519
Registered Nursery1_outside sources	82	0	0	569
Registered Nursery1 (outside sources_root stock)	876	1	0	6976
Registered Nursery 2	487	60	0	2363
Registered Nursery 2 _rootstock	785	0	0	2355
WSDA-collected samples	28	0	0	406
Total	**5289**	**215**	**0**	**28,676**

*Number of single vine samples tested for GLRaV-3. Number of virus positive samples are shown in parenthesis.

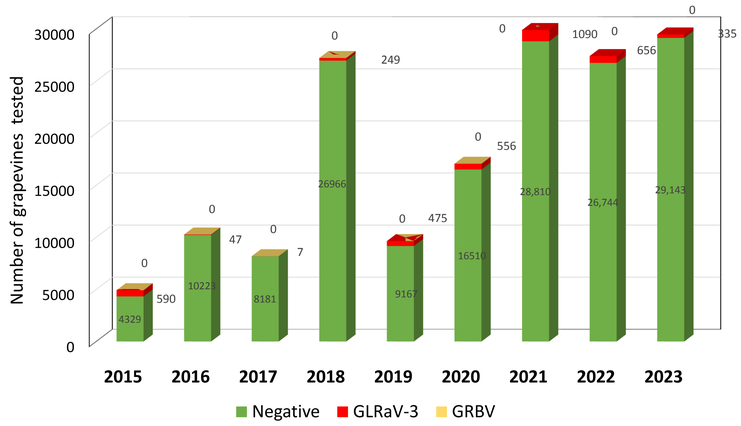


Fig. 2. Summary of RT-PCR/PCR test results carried out between 2015 and 2023 seasons for the presence, respectively, of GLRaV-3 and GRBV in samples collected from registered mother blocks.

Summary and Conclusions

- Continuous monitoring of registered mother blocks is critical to maintain virus-tested grapevines in these blocks. This will help growers to source virus-tested planting stock (own-rooted and grafted vines) for establishing healthy vineyards.
- Like in previous seasons, GRBV was not detected in grapevine samples tested during 2023 (Table 1).
- GLRaV-3 was found at low incidence in registered mother blocks (Fig. 2).
- Testing a subset of samples for GLRaV-1 and GLRaV-4, and the Pierce disease bacterium indicated their absence in samples tested during 2023.
- Annual testing of grapevines and timely sharing of results with concerned nurseries are critical to maintain healthy grapevines in registered nurseries.

Acknowledgements

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