Accepted for publication 19 May 2012. Published 5 August 2013.

A Survey for Grapevine Viruses in Washington State 'Concord' (*Vitis* × *labruscana* L.) Vineyards

Brian W. Bahder, Ph.D. Student, Department of Entomology, **Olufemi Alabi,** Post Doctoral Researcher, Department of Plant Pathology, **Sudarsana Poojari,** Ph.D. Student, Department of Plant Pathology, **Douglas B. Walsh,** Professor, Department of Entomology, and **Rayapati A. Naidu,** Associate Professor, Department of Plant Pathology, Washington State University, Prosser, WA 99350

Corresponding author: Brian W. Bahder. brian.bahder@wsu.edu

Bahder, B. W., Alabi, O., Poojari, S., Walsh, D. B., and Naidu, R. A. 2013. A survey for grapevine viruses in Washington State 'Concord' (*Vitis* × *labruscana* L.) vineyards. Online. Plant Health Progress doi:10.1094/PHP-2013-0805-01-RS.

Abstract

Within the United States, Washington is the number one producer of 'Concord' grapes and the number two producer of wine grapes. Previous studies in wine grapes revealed the presence of twelve different viruses in Washington vineyards. Despite what is known about viruses in wine grapes in Washington, virtually no studies have focused on viruses in Concord vineyards. To further understand the epidemiology of viruses in Washington State, a survey of Concord grapes was conducted in 2010 and 2011. Five hundred Concord vines were sampled and tested for the presence of virus, and five different viruses were found among 92 virus-positive samples. The most common virus found was *Grapevine leafroll-associated virus 3* (51 samples), followed by *Grapevine fanleaf virus* (24 samples), *Grapevine leafroll-associated virus 4* (nine samples), *Grapevine leafroll-associated virus 2* (four samples), and *Grapevine leafroll-associated virus 9* (four samples). The presence of viruses in Concord vineyards is of great concern for growers due to likely movement of virus diseases from Concord grapes to wine grapes that might result in economic losses to the grape industry.

Introduction

Within the United States, Washington is the number one producer of juice grapes (*Vitis* × *labruscana* L. 'Concord' and *Vitis labrusca* 'Niagara') and the number two producer of wine grapes (15). The grape and wine industry have an economic impact of about $8.6 billion at the state level and about $14.9 billion at the national level (23). With a role this large in the local and national economy, the grape industry has a very low tolerance for threats to its sustainability, particularly arthropod pests and pathogens.

There are many different viruses and other graft-transmissible agents reported from all of the major grape-growing regions in the world. To date, twelve different viruses have been detected in Washington vineyards (Table 1) (15). Of these, Grapevine leafroll-associated viruses (GLRaVs) 1, 2, 3, 4, 5, and 9 have been documented in grapevines (*Vitis vinifera*) affected with grapevine leafroll disease (GLRD). This disease is considered the most devastating viral disease of *V. vinifera* cultivars (16) and is present in all major grape-growing regions in the world (10,12). GLRD accounts for 50-60% of global yield losses due to all grapevine viruses and is responsible for about 5-10% of the yield loss in Washington State (16). Of the six GLRaVs known from Washington, *Grapevine leafroll-associated virus 3* (GLRaV-3) was found to be the predominant virus in Washington vineyards (11,16). Grapevine fanleaf disease caused by *Grapevine fanleaf virus* is another very serious viral disease of grapevines. It is cosmopolitan in distribution and can cause up to 80% yield loss in wine grapes, depending on the cultivar (4). Although much information is available on viruses in *V. vinifera* cultivars, comparatively little is known about viruses in grape cultivars native to North America, principally Concord and Niagara (11,12). Recently, GLRaV-3 was documented in Concord and Niagara

grapes in Washington State (22). In contrast to wine grape cultivars, Concord and Niagara cultivars appear to have latent infections of GLRD. With latent infections of GLRD in these cultivars, the prevalence of the disease and its economic impact have received little attention.

Table 1. Grapevine viruses reported from Washington State vineyards.

Virus	Year	Accession No.	Reference
GLRaV-1	2005	Not available	Martin et al. 2005 (11)
GLRaV-2	2005	Not available	Martin et al. 2005 (11)
GLRaV-3	2005	DQ780885	Martin et al. 2005, Soule et al. 2006 (11,22)
GLRaV-4	2006	Not available	Naidu et al. 2011 (15)
GLRaV-5	2006	Not available	Naidu et al. 2011 (15)
GLRaV-9	2008	EF101737	Jarugula et al. 2008*
GRSPaV	2005	FJ943414	Martin et al. 2005, Alabi et al. 2010* (11,)
GV-A	2010	Not available	Naidu et al. 2011 (15)
GV-B	2010	Not available	Naidu et al. 2011 (15)
GFLV	2008	EU573307	Mekuria et al. 2008* (11)
GSyV-1	2010	GU372349	Mekuria and Naidu 2010* (11)
GFkV	2010	AJ309022	Naidu and Mekuria 2010* (11)

* Represents direct submissions to GenBank.

Other important aspects of grapevine virus epidemiology that have been largely ignored in Concord and Niagara vineyards are vector biology and control. The grape mealybug (*Pseudococcus maritimus* Ehrhorn) and the European fruit lecanium scale (*Parthenolecanium corni* Bouché) are known vectors of GLRaV-3 and GLRaV-1 in wine grapes (7,9), but their role in transmitting viruses in juice grape vineyards is unknown. Both vector species are present in Washington vineyards (8). A common occurrence in Washington State is the close proximity of Concord vineyards to wine grape vineyards, and sometimes, the two types of grapes are grown adjacent to each other. Because of the presence of GLRaV-3 in Concord vineyards, the close proximity of Concord vineyards to wine grape vineyards, and the presence of two known vector species in both types of vineyards, it is possible that Concord vineyards could be serving as reservoirs for infection of wine grape vineyards with GLRaV-3 or other viruses, depending on the presence of the appropriate vectors.

The primary objective of this study was to survey Concord vineyards in Washington State to document the presence of grapevine viruses. By knowing exactly what viruses are present in Concord grapes, a better assessment can be made of the epidemiology of grapevine viruses in Washington State vineyards and the risk they pose to grape production.

Collection of Plant Samples

A total of 500 samples were collected between 2010 and 2011 from twelve different Concord vineyard blocks near Prosser, WA. In each vineyard block, 42 Concord vines were sampled randomly. Five leaves, proximal to main trunk and cordon, were detached at the base of the petiole, placed in sealed plastic bags and transported to the Washington State University-Irrigated Agriculture Research and Extension Center, Prosser, for analysis.

Detection of Viruses

Extraction of samples was accomplished using the protocol by Rowhani et al. (2000) (20) by grinding 25 g of petiole tissue in 5 ml of general extraction buffer (GEB; 1.59 g/liter Na_2CO_3, 2.93 g/liter $NaHCO_3$, 2% polyvinyl pyrrolidone, 0.2%

bovine serum albumin, 0.5% Tween 20). Extract was stored at -80°C until further analysis. To extract viral nucleic acid, 4 µl of plant extract was denatured in 25 µl of GES solution with 1% mercaptoethanol at 95°C for 10 min. Denatured product was then held in ice for 5 min. To detect virus in each sample, single-tube reverse transcription (RT)-PCR assays were performed for each virus and for each sample. Forward and reverse primers for each virus are presented in Table 2. RT-PCR conditions for amplification of virus-specific sequences were: initial heating at 52°C for 60 min, initial denaturation at 94°C for 30 s followed by 35 cycles of melting at 94°C for 30 s, annealing at 55°C for 45 s, elongation at 72°C for 30 s, and a final extension at 72°C for 7 min. Five microliters of PCR product was resolved on 1.5% agarose gel stained with GelRed (Biotium, Hayward, CA) and DNA bands were visualized by exposing gels to UV-transilluminator Biorad Universal Hood (Bio-Rad Laboratories, Hercules, CA). Samples that demonstrated successful amplification of desired gene product were either sequenced directly from the PCR product or cloned using TOPO TA Cloning kits with PCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA). All samples were sent to ELIM Biopharmaceuticals Inc. (Hayward, CA) for sequencing. Sequence data were analyzed using Vector NTI (Life Technologies, Grand Island, NY) and MEGA 5 (24).

Table 2. Primers used to test for presence of viruses

Species	Size	Primer name	Sequence (5′-3′)*
GLRaV-1	401	LR1-hsp401/F	GTTACGGCCCTT TGTTTATTATGG[17]
		LR1-hsp401/R	CGACCCCTTTATTGTTTGAGTATG[17]
GLRaV-2	331	LR2-2332/F	ATAATTCGGCGTACATCCCCACTT[17]
		LR2-hsp332/R	GCCCTCCGCGCAACTAATGACAG[17]
GLRaV-3	546	LR3-hsp70/F	CGCTAGGGCTGTGGAAGTATT[19]
		LR3-hsp70/R	GTTGTCCCGGGTACCAGTTAT[19]
GLRaV-4	371	LR4/5-Unl370/F	GGTATGAACAARTTCAATGC[17]
		LR4/5-Unl370/R	TAGACAACCATGTAYTCTATG[17]
GLRaV-5	272	LR5-hsp273/F	AACACTCTGCTTTTCTGCTGGCA[17]
		LR5-hsp273/R	TCTCCAGAAGACGGACCAATGTAA[17]
GLRaV-6	NA	NA	NA
		NA	NA
GLRaV-7	NA	NA	NA
		NA	NA
GLRaV-9	393	LR9/F	CGGCATAAGAAAAGATGGCAC[3]
		LR9/R	TCATTCACCACTGCTTGAAC[3]
GLRaV-10	NA	NA	NA
		NA	NA
GFLV	322	GFLV-2231/F	ACCGGATTGACGTGGGTGAT[21]
		GFLV-2533/R	CCAAAGTTGGTTTCCCAAGA[21]
Species	**Size**	**Primer Name**	**Sequence (5′-3′)***
GFkV	530	GFkV-585/F	CTCAGCCTCCACCTTGCCCCGT[18]
		GFkV-1117/R	CAATTTGGCTGGGCGAGAAGTACA[18]
GSyV-1	298	GSyV-1-Det/F	CAAGCCATCCGTGCATCTGG[2]
		GSyV-1-Det/R	GCCGATTTGGAACCCGATGG[2]
GRSPaV	332	RSP-CP52/F	TGAAGGCTTTAGGGGTTAG[14]
		RSP-CP53/R	CTTAACCCAGCCTTGAAAT[14]
GVA	430	GVA-CP6356/F	GATACYCTAGTTATGCCAGA[13]
		GVA-CP7096/R	GCACCACACTTACACACATTC[13]
GVB	459	GVB-1544/F	GTGCTAAGAACGTCTTCACAGC[13]
		GVB-1983/R	ATCAGCAAACACGCTTGAACC[13]
GVD	465	D_ORF/F	CTTAGGACGCTCTTCGGGTACA[1]
		D_ORF/R	CTGCTCTCCAACCGACGACT[1]

* Superscripts after sequence data represent reference citation for primers original source.

Viruses Present in Concord Vineyards

Ninety-two of the 500 Concord samples tested positive for the presence of virus (Table 3). In this survey, five different viruses were found in Concord grapevines. They are GLRaV-2 (KC110862), GLRaV-3(KC113195-97), GLRaV-4 (KC113198), GLRaV-9 (KC113199), and GFLV (KC113200). Nucleotide identity of virus sequence data among and between vineyards for each virus detected is presented in Table 4. Individual vineyard data are presented in Table 5.

Table 3. Samples tested positive for grapevine viruses.

Virus	Number of samples	Proportion of positive samples	Vineyards
GLRaV-2	4/500 (0.8%)	4.3%	9
GLRaV-3	51/500 (10.2%)	55.4%	1-12
GLRaV-4	9/500 (1.8%)	9.8%	6
GLRaV-9	4/500 (0.8%)	4.3%	8
GFLV	24/500 (4.8%)	26.1%	1, 3, 4, 7

Table 4. Sequence identity between virus samples.

Virus	Sequence identity	Differing base pairs
GLRaV-2	100%	0/545
GLRaV-3	99%	3/545
GLRaV-4	100%	0/450
GLRaV-9	100%	0/500
GFLV	100%	1/322

Table 5. Vineyard information and viruses detected.

Vineyard	Viruses present (no. of samples)	Size (ha)	Age (years)
1	GLRaV-3 (7), GFLV (10)	40	5
2	GLRaV-3 (3)	35	6
3	GLRaV-3 (4), GFLV (3)	45	7
4	GLRaV-3 (2), GLFV (2)	50	6
5	GLRaV-3 (2), GLRaV-9 (4)	30	8
6	GLRaV-3 (1), GLRaV-4 (9)	25	15
7	GLRaV-3 (4), GFLV (9)	35	7
8	GLRaV-3 (9)	30	7
9	GLRaV-3 (2), GLRaV-2 (4)	15	5
10	GLRaV-3 (1)	15	2
11	GLRaV-3 (12)	30	6
12	GLRaV-3 (3)	20	8

This survey establishes new records for the presence of GLRaV-2, GLRaV-4, GLRaV-9, and GFLV in Concord vineyards in Washington State. Owing to a wide distribution of GLRaV-3 in grape growing regions and the established presence of GLRaV-3 in Concord grapes in Washington, the presence of other GLRaVs was not unexpected. Also, GLRaV-3 was demonstrated to be the predominant virus encountered in Concord grapes in this survey, which is in congruence with many other surveys (6,10,11,12,22,25). The sample D2 appeared to have very similar sequence to the isolate WA C1-1 (DQ780885) previously documented from Concord grapes (22). Sequence data for the heat shock protein subunit 70 (hsp-70) region of GLRaV-3 obtained from other samples, however, appeared to be more similar to the GLRaV-3 isolate 4448 (HM636877), and isolate known from *V. vinifera* cv. 'Crimson seedless' in Chile. The presence of isolates in *V.* × *labruscana* L. similar to GLRaV-3 isolates known from *V. vinifera* in Washington is an indicator that GLRaV-3 could potentially be spreading from *V. vinifera* to *V.* × *labruscana* L. To date, the presence of isolates similar to those from *V.* × *labruscana* L. in *V. vinifera* has not been demonstrated. The

occurrence of GLFV is interesting because the natural vector, *Xiphinema index*, has never been recorded from Washington State. It is likely that infected, propagative materials used to establish vineyards originated from a single nursery source, as indicated by identical sequence data for all GFLV samples in the study.

In Washington State, many Concord vineyards are grown adjacent to wine grape vineyards. This practice may be allowing Concord vineyards to serve as a reservoir for infecting *V. vinifera* vineyards with GLRaVs. While Concord grapes may be a source of inoculum for GLRaVs to be spread into wine grapes, the original source of GLRaVs was most likely the arrival of infected propagation materials from other grape growing regions and has consequently moved into Concord vineyards. All vineyards in this survey had established populations of *Ps. maritimus* (5) and *Pa. corni* were observed in each Concord vineyards sampled. One vine that tested positive for GLRaV-3 also had *Pa. corni* on its trunk. Currently, only *Ps. maritimus* is known to vector GLRaV-3 between *V. vinifera* plants with no data on their ability to vector the virus between *V.* × *labruscana* L. or between *V. vinifera* and *V.* × *labruscana* L.

Management practices aimed at infection and vector control are minimal in *V.* × *labruscana* L. because of the apparent lack of symptoms and economic impact. The lack of control of GLRD in Concord vineyards, the presence of GLRaVs in many Concord vineyards, and the presence of known vectors of GLRaVs in these vineyards create an increased risk to the sustainability of wine grape vineyards that are in close proximity. This threat highlights a clear necessity for future work to focus on the vector capabilities of *Pa. corni* and *Ps. maritimus* with regard to interspecific transmission of GLRaV-3 in grapes in Washington State.

Acknowledgments

Funding for this project was provided by the USDA-NIFA Specialty Crop Research Initiative 2009-02486, Concord Research Council, Washington Wine Advisory Committee, and the Washington State Commission on Pesticide Registration. Vineyards sampled were provided by Hogue Ranches (Prosser, WA) and Wyckoff farms. PPNS number 0618, Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences, Agricultural Research Center Project Number WNPO 0616, Washington State University, Pullman, WA 99164-6240.

Literature Cited

1. Abou-Ghanem, N., Saldarelli, P., Minafra, A., Buzkan, N., Castellano, M. A., and Martelli, G. P.1997. Properties of Grapevine virus D, a novel putative Trichovirus. J. Plant Pathol. 78:15–25.
2. Al Rwahnih, M., Daubert, S., Golino, D., and Rowhani, A. 2009. Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveal a multiple virus infection that includes a novel virus. Virology 387:395-401.
3. Alkowni, R., Rowhani, A., Daubert, S., and Golino, D. 2004. Partial characterization of a new ampelovirus associated with Grapevine leafroll disease. J. Plant Pathol. 86:123-133.
4. Andret-Link, P., Laporte, C., Valat, L., Ritzenhaler, C., Demangeat, G., Vigne, E., Laval, V., Pfeiffer, P., Stussi-Garaud, C., and Fuchs, M. 2004. *Grapevine fanleaf virus*: still a major threat to the grapevine industry. J. Plant Pathol. 86:183-195.
5. Bahder, B. W., Naidu, R. A., Daane, K. M., Millar, J. G., and Walsh, D. B. 2012. Pheromone-based monitoring of *Pseudococcus maritimus* (Hemiptera: Pseudococcidae) populations in Concord grape vineyards. J. Econ. Entomol. 106:482-490.
6. Fuchs, M., Martinson, T. E., Loeb, G. M., and Hoch, H. C. 2008. Survey for the three major leafroll disease-associated viruses in finger lakes vineyards in New York. Proc. of the 2nd Annual Natl. Viticulture Res. Conf. Agriculture and Natural Resources, University of California. Davis, CA.
7. Golino, D. A., Sim, S. T., Gill, R., and Rowhani, A. 2002. California mealybugs can spread grapevine leafroll disease. Calif. Agric. 56:196-201.

8. Grime, C. and Cone, W. W. 1985. Control of the grape mealybug, *Pseudococcus maritimus* (Ehrhorn)(Homoptera: Pseudococcidae), on Concord Grapes in Washington. J. Entomol. Soc. B. C. 82: 3- 6.
9. Hommay, G., Komar, V., Lemaire, O., Herrbach, E. 2008. *Grapevine virus A* transmission by larvae of *Parthenolecanium corni*. Eur. J. Plant Pathol. 121:185-188.
10. MacKenzie, D. J., Johnson, R. C., and Warner, C. 1996. Incidence of four important viral pathogens in Canadian vineyards. Plant Dis. 80:955-958.
11. Martin, R. R., Eastwell, K. C., Wagner, A., Lamprecht, S., and Tzanetakis, I. E. 2005. Survey for viruses of grapevine in Oregon and Washington. Plant Dis. 89:763-766.
12. Milkus, B. N., and Goodman, R. N. 1999. A survey of Missouri vineyards for the presence of five grape viruses. Am. J. Enol. Vitic. 50:133-134.
13. Minafra, A., Saldarelli, P., Grieco, F., and Martelli, G. P. 1994. Nucleotide sequence of the 3' terminal region of the RNA of two filamentous grapevine viruses. Arch. Virol. 137:249-261.
14. Lima, M. F., Alkowni, R., Uyemoto, J. K., Golino, D., Osman, F., and Rowhani, A. 2006. Molecular analysis of a California strain of Rupestris stem pitting-associated virus isolated from declining Syrah grapevines. Arch. Virol. 151:1889-1894.
15. Naidu, R. A. 2011. Virus update: The status of Washington vineyards. Viticulture and Enology Extension News Fall 2011.
16. Naidu, R. A., O'Neal, S., and Walsh, D. B. 2008. Grapevine leafroll disease. Ext.Publ. EB2027E. Washington State Univ., Prosser, WA.
17. Osman, F., Leutenegger, C., Golino, D., and Rowhani, A. 2007. Real-time RT-PCR (TaqMan) assays for the detection of Grapevine leafroll associated viruses 1-5 and 9. J. Virol. Methods 141:22-29.
18. Osman, F., Leutenegger, C., Golino, D., and Rowhani, A. 2008. Comparison of low-density arrays, RT-PCR and real-time TaqMan RT-PCR in detection of grapevine viruses. J. Virol. Methods 149:292-299.
19. Osman, F., and Rowhani, A. 2006. Application of a spotting sample preparation technique for the detection of pathogens in woody plants by RT-PCR and real-time PCR (TaqMan). J. Virol. Methods 133:130-136.
20. Rowhani, A., Biardi, L., Johnson, R., Saldarelli, P., Zhang, Y.P., Chin, J. and Green, M. 2000. Simplified sample preparation method and one-tube RT-PCR for grapevine viruses. 13th ICGV, Extended Abstracts. Proc. of XIII Intl. Council for the Study of Viruses and Virus-Like Dis. of the Grapevine, Adelaide, Australia.
21. Sanchez, F., Chay, C., Borja, M. J., Rowhani, A., Romero, J., Bruening, G., Ponz, F. 1991. cDNA sequence of the capsid protein gene and 3 untranslated regions of a fanleaf isolate of *Grapevine fanleaf virus*. Nucleic Acid Residues 19:5440.
22. Soule, M. J., Eastwell, K. C., and Naidu, R. A. 2006. First Report of *Grapevine leafroll associate virus-3* in American *Vitis* spp. grapevines in Washington State. Plant Dis. 90:1461.
23. Stonebridge Research Group LLC. 2012. The economic impact of Washington State wine and grapes. Stonebridge Research Group LLC., 105b Zinfandel Lane, St. Helena, CA. www.stonebridgeresearch.com.
24. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731-2739.
25. Walker, J. T. S., Charles, J. G., Froud, K. J., and Connolly, P. 2004. Leafroll virus in vineyards: Modeling the spread and economic impact. HortResearch Client Report No. 12795. The Horticulture and Food Research Institute of New Zealand, Auckland, NZ.