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Influence of grapevine leafroll associated viruses (GLRaV-2 and -3) on the fruit composition of Oregon *Vitis vinifera* L. cv. Pinot noir: Phenolics

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ABSTRACT

Some of the 10 known grapevine leafroll associated viruses (GLRaVs) have negative impacts upon vine productivity and grape quality, though these negative influences are dependent on factors such as GLRaV strain, cultivar, clone, rootstock, and vine age. This is the first study to report on GLRaV-2 and GLRaV-3 infected vines, with regard to phenolic compounds, and other fruit maturity indices, of 'Pinot noir' grapes, compared to berries from adjacent vines free of GLRaVs (same vineyards). Three different rootstock/scion combinations were included in this study. Clusters were collected for two growing seasons from commercial vineyards in the Willamette Valley of Oregon, and each vine sampled was tested for GLRaV-1, -2, -3 and Rupestris stem pitting-associated virus (RSPaV). All sampled vines were infected with RSPaV. Grapevine leafroll associated virus-infected vines tested positive for GLRaV-2 or GLRaV-3. Overall, fruit infected with GLRaV-2 and -3 had reduced percent soluble solids, decreased individual and total anthocyanins, and increased skin and pulp weight for all three 'Pinot noir' rootstock/scion combinations examined. *Vitis riparia* rootstock/'Pinot noir' clone 114 scion combination appeared to be the most sensitive to GLRaV-3 infection, having significant reduction of all five anthocyanins, total phenolics, and total tannins, with an increased cluster weight and 100-berry weight. No clear trends were observed in the polyphenolics analysed.

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1. Introduction

Oregon's wine and wine grape industry makes a large economical contribution to the state (>$1.4 billion dollars in 2006; Oregon Wine Board). Wine grapes are a high-value fruit crop and Oregon is an important contributor to wine grape production in the United States (National Agricultural Statistics Service, 2007). Grapevine leafroll associated viruses (GLRaVs) have been recently confirmed in vineyards located within Oregon (Martin, Eastwell, Wagner, Lamprecht, & Tzanetakis, 2005). The virus has also been reported to negatively affect vine performance (mainly productivity) and grape quality (Cabaleiro, Segura, & Garcia-Berrios, 1999; Guidoni, Mannini, Ferrandino, Argamante, & Di Stefano, 1997; Kliewer & Lider, 1976; Kovacs, Hanami, Fortenberry, & Kaps, 2001; Wolpert & Vilas, 1992). A recent survey of Oregon and Washington vineyards demonstrated that less than 5% of vines were infected with GLRaV-1, -2, or -3 (Martin et al., 2005). The infection rate was low, but GLRaV-3 has been demonstrated to spread rapidly in other wine-growing regions once established, with an infection increase of 8–12% per year reported in a Spanish vineyard (Cabaleiro & Segura, 2006). There are numerous viruses associated with this disease, hence the name GLRaV (Martelli et al., 2002; Prosser, Goszcynski, & Meng, 2007). Previous studies have reported on the reduced performance (i.e. lower vigor, decreased yield, delayed and uneven ripening, reduced berry pigmentation, and reduced sugar accumulation) of grapevines infected with GLRaVs (Cabaleiro et al., 1999; Guidoni et al., 1997; Kliewer & Lider, 1976; Kovacs et al., 2001; Wolpert & Vilas, 1992). Cultivar, clone, rootstock, vine age, specific GLRaV and mixed infections are a few factors that influence the symptoms and their severities observed caused by GLRaV infection (Cabaleiro et al., 1999; Golino, 1993; Guidoni et al., 1997; Kliewer & Lider, 1976; Kovacs et al., 2001; Krake, 1993; Wolpert & Vilas, 1992).

Phenolics are secondary plant metabolites that contribute to the colour (appearance), taste (bitterness), and texture (astringency) of red wine, and have exhibited potential benefits to human health (Cheynier, 2005). Colour (i.e., anthocyanins) is an important quality indicator of red grapes and wine, and is impacted by biotic stresses such as pathogen attack (Gould & Lister, 2006). Few scientific reports have been published on how GLRaV impacts fruit quality qualitatively or quantitatively (Cabaleiro et al., 1999; Guidoni et al., 1997; Kovacs et al., 2001; Krake, 1993), and the majority of those that have dealt with GLRaV focused on its spread, occurrence, or detection methods (Golino, Sim, Gill, & Rowhani, 2002; Martin et al., 2005; Osman, Leutenegger, Golino, & Rowhani,

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2007). It is known that GLRaV is known to have an impact on colour development in red grape cultivars (Guidoni et al., 1997; Krake, 1993) but there are no papers, to the best of our knowledge, that have reported on how GLRaV influences the anthocyanin profile of 'Pinot noir' grapes. 'Pinot noir' is the most important and abundant (>17,000 tons harvested in 2006 in Oregon) and significant cultivar grown in the Willamette Valley of Oregon (Martin et al., 2005; Oregon Wine Board).

The objective of this study was to compare the biochemical components (i.e. anthocyanins, phenolics other than anthocyanins, and simple fruit maturity indices) of 'Pinot noir' grapes from vines infected with or free of GLRaV-2 or -3, to better understand how GLRaV affects 'Pinot noir' grape quality in commercial fields.

2. Materials and methods

2.1. Plant material

Vineyard locations, vine information, and harvest dates are shown in Table 1. Vineyard one (VY1) was located near Amity, Oregon and the second vineyard (VY2) site was located near Yamhill, Oregon, approximately 40 km apart. VY2 had two types of vines; rootstock/scion information is in Table 1 (VY2a and VY2b). VY1 and VY2 were both commercially operated vineyards.

Clusters were taken from randomly selected, paired vines located in the two vineyards, and initially visually categorised as having GLRaV foliar symptoms (assumed naturally infected) or not having visual symptoms (assumed healthy vines). The individual vines, or in some cases clusters, were tested to confirm the presence or absence of GLRaV-2 or -3 in each plant used for fruit analyses. Initial testing showed GLRaV-2 was present in VY1, and GLRaV-3 predominated in VY2, with a low incidence of GLRaV-2, while GLRaV-1 and GLRaV-4 through GLRaV-9 were not detected. Rupestris stem pitting-associated virus (RSPaV) was present in both vineyards.

Vineyards' harvest dates were decided by vineyard managers and winemakers. Sampling frequency and other details are in Table 1. Samples were collected for two growing seasons (2005 and 2006). Four clusters were collected from each selected vine and biochemical analyses were performed on a per vine basis. Clusters were collected from different random paired vines at each sampling period, to avoid the impact of cluster removal. Clusters from one vine were grouped for chemical analysis.

2.2. Reagents, chemicals, and standards

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless specified otherwise. Solvents and chemicals for this investigation were analytical and high performance liquid chromatography (HPLC) grade. Malvidin glucoside was purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ).

2.3. Virus detection

Sample collection and RT-PCR virus tests were done as described previously (Martin et al., 2005), with the exception that RNA was extracted as described by Spiegel and Martin (1993). Results of the virus testing are presented in Table 1.

2.4. Cluster weight, berry weight, fractionation, and extraction

Clusters were stored at −23 °C until processing. Each sample's cluster and berry weight was recorded. One hundred berries were randomly selected, weighed, and fractionated into two portions (skins and pulp: fraction **a**; seeds: fraction **b**) while they were still frozen, and these fractions were immediately placed in liquid nitrogen (Norco Inc., Nampa, ID). Excess liquid nitrogen was evaporated off, and fraction weights were recorded. Frozen fractions were stored at −80 °C until extraction. Extraction was performed as described in Lee and Finn (2007) with the following changes: IKA M20 Universal mill (IKA Works Inc., Wilmington, NC) was used to powder fraction **a** in liquid nitrogen. Fraction **b** was extracted as whole seeds. All fractions were extracted and re-extracted two more times (extracted a total of three times) in acidified MeOH (0.1% formic acid, v/v). These extracts were then evaporated under vacuum at 40 °C using a RapidVap Vacuum Evaporation System (Labconco Corp., Kansas City, MO) and re-dissolved in water (final volume of 25 ml). Aqueous extracts (stored at −80 °C until analysis) were used for all phenolic analyses.

2.5. Fruit maturity indices

Whole berries were puréed and centrifuged for 10 min at 4000 rpm, and the supernatant was used for measurements of % soluble solids. Straight purée was used to measure pH and titratable acidity (TA). Percent soluble solids, TA, and pH measurements were conducted as described in Lee and Finn (2007) with the exception of a Mettler-Toledo autotitrator T50A (Mettler Toledo Inc., Columbus, OH) for TA determination, and samples were titrated to an end point of pH 8.2. TA was expressed as tartaric acid equivalents.

2.6. Total anthocyanins (TACY), total phenolics (TP), and total tannins (TT) determination

A SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA) and clear 96-well flat-bottom plates (Nalge Nunc International, Rochester, NY) were used for all three measure-

Table 1
Description of the samples collected from two vineyards

	Vineyard 1	Vineyard 2	Vineyard 2
Code	VY1	VY2a	VY2b
Location	Amity, OR	Yamhill, OR	Yamhill, OR
Cultivar	Pinot noir	Pinot noir	Pinot noir
Rootstock/scion	Unknown rootstock/'Chardonnay' interstock/'Pinot noir' scion (clone unknown)	*V. riparia* rootstock/'Pinot noir' clone 114	Self-rooted/'Pinot noir' clone Pommard
Virus tested for	GLRaV types 1–3 and RSPaV	GLRaV types 1–3 and RSPaV	GLRaV types 1–3 and RSPaV
Virus confirmation	GLRaV-2 and RSPaV	GLRaV-3 and RSPaV	GLRaV-3 and RSPaV
Sampling dates for both seasons (code used in Figs. 1 and 2)	VY1-1: 9/19/2005 VY1-2: 9/26/2005 VY1-3: 10/3/2005 VY1-4: 10/11/2005 VY1-5: 10/17/2005 VY1-6: 9/15/2006 VY1-7: 9/22/2006 VY1-8: 9/28/2006	VY2a-1: 9/28/2005 VY2a-2: 10/3/2005 VY3a-2: 9/15/2006 VY2a-4: 9/22/2006	VY2b-1: 9/28/2005 VY2b-2: 10/3/2005 VY2b-3: 9/15/2006 VY2b-4: 9/22/2006 VY2b-5: 9/28/2006

ments. TACY was determined as described in Lee, Durst, and Wrolstad (2005), with the following modifications: absorbance was measured at 520 and 700 nm, and TACY was expressed as malvidin 3-glucoside (molar extinction coefficient of 28,000 $l\ cm^{-1}\ mol^{-1}$ and molecular weight of 493.3 $g\ mol^{-1}$). TP were expressed as gallic acid, and TT were expressed with epicatechin standards. Both of these methods were described in detail by Waterhouse (2002) and Sarneckis et al. (2006). All three measurements were conducted in duplicate.

2.7. HPLC/DAD and HPLC/DAD/ESI-MS/MS analyses of anthocyanins and other polyphenolics

Extracts were analysed as described in Lee and Finn (2007), with an alteration in injection volumes (10 μl for anthocyanin analysis and 40 μl for the non-anthocyanin polyphenolics). Briefly, an HP1100 system equipped with a DAD and XCT ion trap mass spectrometer (Agilent Technologies Inc., Palo Alto, CA) was used. HPLC/DAD was used for identification and quantification based on retention time and UV–Vis spectra. HPLC/DAD/ESI-MS/MS was used for identification to obtain molecular and fragmented ions of peaks of interest. Authentic standards were co-chromatographed to identify the compounds when available. Anthocyanins were quantified as malvidin glucoside (external standard) at 520 nm. Phenolic acids were quantified as caffeic acid (320 nm), flavanols as catechin (280 nm), and flavonol glycosides as quercetin rutinoside (370 nm). Phenolics other than anthocyanins will be referred to as polyphenolics in the following sections for conciseness.

2.8. Statistical analysis

Statistica for Windows version 7.1 was used (StatSoft, Inc., Tulsa, OK) for *t*-test calculations and one-way analysis of variance (ANOVA) for infected versus uninfected sample pairs from vines with the same rootstock/scion combination ($\alpha = 0.05$). *t*-Test and one-way ANOVA were determined from values of healthy vines within VY2 (healthy samples from VY2a and VY2b). A correlation was calculated for the total anthocyanin values obtained by the two methods ($\alpha = 0.05$), by spectrophotometer and HPLC.

3. Results and discussion

All vines sampled in this study were found to be infected with RSPaV, which has been reported to alter vine performance, TA, and pH slightly (Reynolds, Lanterman, & Wardle, 1997). Since all vines were RSPaV-positive, the effect of berry composition observed in this study was probably due to GLRaV-2 or -3 co-infection with RSPaV. Samples from VY2a and VY2b were from a non-irrigated vineyard that had visual signs of water stress during both seasons. In VY1, GLRaV-2 was introduced during grafting of a 'Chardonnay' on a rootstock vineyard to a 'Pinot noir' vineyard. The resulting vineyard was a 'Pinot noir' scion on a 'Chardonnay' interstock on a rootstock. VY1 was grafted over a 2-year period and the first year was from an ungrafted 'Pinot noir' vineyard that was infected with GLRaV-2, while the second year the 'Pinot noir' came from a different vineyard that was free of GLRaV. The incidence of GLRaV-2 in VY1 correlated with the grafting sources. VY2 originally had a block of GLRaV-3 infected experimental vines that have since been removed. However, the GLRaV-3 had spread to adjacent vines, suggesting the presence of mealybug vectors. Samples from VY2 were from the adjacent blocks. The two growing seasons data were pooled, since the interaction between virus status and growing seasons were not significantly different.

3.1. Fruit maturity indices, TACY, TP, and TT

Fruit maturity indices are summarised in Table 2. Mean cluster size, weight per 100 berries, fraction weights, pH, and TA trends were different depending on the rootstock/scion combination and sampling location. Plants with GLRaV infection had a decreased cluster size and fraction **b** weight in VY1 and VY2b, but the opposite was observed in VY2a samples. GLRaV-3 positive samples from VY2a had smaller cluster and seed fraction weights (both $p \leqslant 0.05$), compared to the corresponding GLRaV-3 negative samples. Disparity in cluster size observed between VY2a and VY2b samples might also be due to the different rootstock/scion interactions, since healthy clusters from the same location were significantly different as well ($p \leqslant 0.05$). Clusters from healthy VY2a plants were about 40% larger than those from VY2b. Clusters from GLRaV-3 positive 'Nebbiolo' plants have been reported to be smaller than those from heat-treated vines (virus eliminated; Guidoni et al., 1997). GLRaV-infected 'Zinfandel' also had smaller clusters than uninfected plants, but in the case of infected 'Riesling,' larger clusters were found (Wolpert & Vilas, 1992). GLRaV-positive plants from VY1 and VY2a generally had smaller berries than GLRaV-negative samples. Larger berries have been observed in 'Albariño' grapes infected with GLRaV-2 (Cabaleiro et al., 1999), and in 'Riesling' and 'Zinfandel' infected with mild leafroll virus (type not reported;

Table 2
Virus status, weights, fruit maturity indices, and spectrophotometric measurements at harvest for all locations

Sample code from the two vineyards	VY1		VY2a		VY2b	
Harvest dates	17/10/2005 and 28/9/2006		03/10/2005 and 22/9/2006		03/10/2005 and 28/9/2006	
Virus status	GLRaV-2 positive	GLRaV-2 negative	GLRaV-3 positive	GLRaV-3 negative	GLRaV-3 positive	GLRaV-3 negative
Number of vines corresponding to the GLRaV results	7	8	5	11	6	10
Average cluster weight (g)	71 (7) a	86 (6) a	109 (8) a	69 (8) b	69 (7) a	74 (8) a
100 berries weight (g)	113 (3) a	110 (3) a	121 (6) a	112 (3) a	119 (3) a	119 (2) a
Fraction **a** weight (g) per 100 berries	106 (3) a	101 (3) a	111 (5) a	104 (3) a	110 (3) a	109 (2) a
Fraction **b** weight (g) per 100 berries	6 (0.3) a	7 (0.4) a	8 (0.4) a	7 (0.3) b	7 (0.7) a	8 (0.5) a
pH	3.77 (0.03) a	3.74 (0.04) a	3.88 (0.08) a	3.73 (0.04) a	3.77 (0.04) a	3.79 (0.05) a
TA (g of tartaric acid/100g berries)	0.73(0.03) a	0.68 (0.03) a	0.57 (0.01) a	0.58 (0.01) a	0.56 (0.02) a	0.55 (0.01) a
% Soluble solids (°Brix)	24.6 (0.5) a	25.1 (0.6) a	24.7 (0.5) a	25.8 (0.5) a	25.3 (0.7) a	26.8 (0.5) a
TACY (mg of malvidin glucoside/100 g berries)	62.5 (4.6) a	70.6 (3.9) a	44.9 (4.5) a	71.2 (6.1) b	52.1 (3.0) a	56.2 (5.2) a
TP (mg of gallic acid/100 g of berries)	226 (6.4) a	237 (15.4) a	187 (16.2) a	250 (14.6) b	203 (9.2) a	188 (10.9) a
TT of whole berries (mg of epicatechin/100 g of berries)	159 (9.2) a	156 (18.5) a	135 (9.7) a	193 (14.4) b	159 (10.9) a	151 (10.3) a
TT of fraction **a** (mg of epicatechin/100 g of berries)	144 (9.5) a	146 (18.6) a	127 (10.6) a	178 (15.7) a	151 (11.0) a	138 (9.8) a
TT of fraction **b** (mg of epicatechin/100 g of berries)	14.4 (3.0) a	10.4 (2.0) a	7.1 (1.2) a	14.4 (3.9) a	8.0 (1.7) a	12.6 (2.3)a

All sampled vines tested positive for RSPaV. Different lower case letters indicate significantly different ($p \leqslant 0.05$) within the pair of samples (e.g., samples from GLRaV-2 positive versus negative vines from vineyard 1). Values in parentheses are standard errors.

Table 3
Anthocyanins and polyphenolic values expressed as mg/100 g of berries

		λ_{max} (nm)	Molecular and *fragmented ions* (*m*/*z*)	VY1		VY2a		VY2b	
Virus status				GLRaV-2 positive	GLRaV-2 negative	GLRaV-3 positive	GLRaV-3 negative	GLRaV-3 positive	GLRaV-3 negative
Anthocyanin by HPLC									
A1	Delphinidin glucoside	520	465, *303*	4.28 (0.72) a	5.97 (1.41) a	2.19 (0.50) a	5.47 (0.87) b	3.62 (0.64) a	4.57 (1.29) a
A2	Cyanidin glucoside	520	449, *287*	1.27 (0.21) a	1.87 (0.45) a	0.67 (0.12) a	1.56 (0.23) b	1.16 (0.11) a	1.36 (0.26) a
A3	Petunidin glucoside	520	479, *317*	4.60 (0.55) a	5.97 (0.98) a	2.90 (0.47) a	6.15 (0.82) b	4.18 (0.58) a	4.99 (1.09) a
A4	Peonidin glucoside	520	463, *301*	10.4 (0.69) a	13.6 (1.12) b	7.96 (0.97) a	13.5 (1.34) b	8.99 (0.45) a	11.2 (0.53) b
A5	Malvidin glucoside	520	493, *331*	40.3 (2.57) a	43.0 (1.53) a	31.3 (2.82) a	47.3 (3.53) b	35.8 (2.29) a	37.0 (2.68) a
Total anthocyanin				60.8 (3.65) a	70.4 (4.31) a	45.0 (4.59) a	74.0 (6.71) b	53.8 (3.76) a	59.1 (5.51) a
Polyphenolics by HPLC									
Fraction **a** (skins and pulp)									
FA1	Gallocatechin	280	305	Trace	Trace	Trace	Trace	Trace	Trace
FA2	Protocatechuic acid	320	153	0.23 (0.04) a	0.22 (0.05) a	0.05 (0.01) a	0.19 (0.04) b	0.19 (0.09) a	0.11 (0.03) a
FA3	Caftaric acid	320	311, *179*	1.98 (0.43) a	1.85 (0.61) a	0.57 (0.20) a	1.49 (0.35) a	0.89 (0.26) a	0.61 (0.13) a
FA4	Procyanidin 1	280	577, *425*	0.48 (0.08) a	0.40 (0.06) a	0.28 (0.14) a	0.33 (0.05) a	0.31 (0.03) a	0.30 (0.05) a
FA5	Coutaric acid	320	295, *163*	0.38 (0.09) a	0.33 (0.10) a	0.06 (0.01) a	0.20 (0.05) a	0.19 (0.10) a	0.10 (0.02) a
FA6	Catechin	280	289	1.23 (0.07) a	1.15 (0.10) a	1.15 (0.11) a	0.99 (0.15) a	0.77 (0.14) a	0.79 (0.12) a
FA7	Fertaric acid	320	325, *193*	0.05 (0.01) a	0.05 (0.02) a	0.02 (0) a	0.03 (0.01) a	0.02 (0.01) a	0.01 (0) a
FA8	Procyanidin 2	280	577	0.77 (0.10) a	0.70 (0.05) a	1.11 (0.10) a	0.67 (0.08) b	0.65 (0.06) a	0.52 (0.04) a
FA9	Epicatechin	280	289	0.46 (0.10) a	0.65 (0.17) a	0.27 (0.03) a	0.44 (0.12) a	0.35 (0.09) a	0.27 (0.03) a
FA10	Quercetin galactoside	370	463, *301*	0.92 (0.12) a	1.02 (0.11) a	0.66 (0.07) a	1.12 (0.09) b	1.07 (0.12) a	0.86 (0.14) a
FA11	Quercetin glucuronide	370	477, *301*	8.42 (0.66) a	8.65 (0.67) a	8.58 (0.54) a	9.72 (0.70) a	9.67 (0.86) a	7.53 (0.91) a
FA12	Kaempferol glucoside	370	447, *284*	0.80 (0.09) a	0.91 (0.12) a	0.94 (0.07) a	1.21 (0.13) a	1.09 (0.12) a	0.84 (0.11) a
FA13	Isorhamnetin glucoside	370	477, *314*	0.07 (0.01) a	0.08 (0.02) a	0.06 (0.01) a	0.11 (0.02) a	0.05 (0.01) a	0.08 (0.02) a
Total of fraction **a**				15.8 (1.06) a	16.0 (1.74) a	13.8 (0.73) a	16.5 (1.52) a	15.2 (1.31) a	12.0 (1.30) a
Fraction **b** (seeds)									
FB1	Catechin	280	289	3.33 (0.58) a	2.82 (0.56) a	1.54 (0.30) a	2.99 (1.48) a	2.19 (0.30) a	3.32 (0.86) a
FB2	Procyanidin 3	280	577, *407*, *425*	0.23 (0.05) a	0.16 (0.03) a	0.08 (0.01) a	0.20 (0.06) a	0.12 (0.03) a	0.19 (0.03) a
FB3	Epicatechin	280	593, *425*	1.68 (0.30) a	1.36 (0.18) a	0.73 (0.13) a	1.36 (0.31) a	0.86 (0.18) a	1.25 (0.21) a
FB4	(epi)catecin-epicatechin gallate or epicatechin gallate-(epi)catechin	280	593, *425*	0.20 (0.05) a	0.13 (0.03) a	0.05 (0.01) a	0.23 (0.10) a	0.12 (0.03) a	0.18 (0.04) a
FB5	Epicatechin gallate	280	441, *289*	0.18 (0.03) a	0.13 (0.02) a	0.07 (0.01) a	0.20 (0.09) a	0.11 (0.02) a	0.15 (0.03) a
Total of fraction **b**				5.61 (0.96) a	4.60 (0.75) a	2.49 (0.46) a	4.98 (1.03) a	3.40 (0.54) a	5.10 (1.12) a
Total polyphenolics				21.4 (1.04) a	20.6 (1.77) a	16.2 (0.80) a	21.5 (1.59) a	18.6 (1.08) a	17.1 (2.11) a

All sampled vines tested positive for RSPaV. Different lower case letters within a row from each pair were significantly different ($p \leqslant 0.05$). Values in parentheses are standard errors. Peak abbreviations listed before individual phenolics were used in Figs. 1 and 2. Details of VY1, VY2a, and VY2b are listed in Table 1.

Wolpert & Vilas, 1992), but berries were smaller in 'St. Vincent' and 'Vidal blanc' grapes infected with GLRaV-3 (Kovacs et al., 2001). Fraction **a** (skins and pulp) weight was greater in all plants that were GLRaV-positive versus those testing negative. Fraction **b** (seeds) weighed more in GLRaV-positive plants from VY2a, but the opposite was observed in VY1 and VY2b samples.

The pH, TA, and percent soluble solids were all statistically similar among the pair means (Table 2), but general trends are pointed out. The pH was slightly higher in infected grapes from VY1 and VY2a, but not in VY2b (Table 2). TA was slightly higher in infected grapes from VY1 and VY2b, but not in VY2a (Table 2). In past reports, higher or lower TA values in infected versus healthy vines depended on cultivar examined and season, and was either significant or not significant within the cultivar tested, indicating vast responses to virus infection (Cabaleiro et al., 1999; Kliewer & Lider, 1976; Wolpert & Vilas, 1992). TA values were overall lower in grapes from VY2 (both a and b) than grapes from VY1 (Table 2), which was probably due to VY2 being a non-irrigated site. Lower TA in grapes at harvest from non-irrigated sites has been observed by Esteban, Villanueva, and Lissarrague (2002). Non-irrigated vineyards are normally not water-limited in Oregon, but during 2005 and 2006, vines at VY2 were visually water-stressed. Percent soluble solids for GLRaV-infected grapes were numerically slightly lower than from their healthy counterparts ($\leqslant$1.5 °Brix difference, Table 2), which has also been reported in other cultivars such as 'St. Vincent', 'Vidal blanc', 'Albariño', 'Riesling', and 'Zinfandel' (Cabaleiro et al., 1999; Guidoni et al., 1997; Kovacs et al., 2001; Wolpert & Vilas, 1992).

Spectrophotometric measurements are summarised in Table 2. TACY was numerically higher in all samples from healthy vines compared to infected vines but the difference was only significant when contrasting those samples from VY2a. TP were similar in VY1 and VY2b but lower in VY2a-infected samples, compared to their healthy counterparts. TT was significantly lower for whole berry samples from infected VY2a vines. Fruit maturity indices, TP, and TT trends varied, depending on the rootstock/scion combination. VY2a healthy berries had greater TT for both fractions, when compared to VY2b healthy berries. GLRaV-3 positive fraction **b** TT levels were similar for both rootstock/scion combinations from VY2 (decreased TT in fraction **b**), but changed in fraction **a** TT levels (decreased in VY2a samples, but increased in VY2b samples). Only samples from VY2a showed statistical difference in cluster weights, fraction **b** weights, TACY, TP, and TT, which implied this rootstock/scion combination was more sensitive to GLRaV infection.

3.2. Anthocyanins by HPLC

Five individual anthocyanins (glucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin; Table 3) were identified in 'Pinot noir' berries, as also reported by numerous others (Cortell, Halbleib, Gallagher, Righetti, & Kennedy, 2007; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999; Price, Breen, Valladao, & Watson, 1995). For VY1 and VY2b the grapes from the paired uninfected and infected vines were not different for any anthocyanin or polyphenolic values, with the exception of peonidin glucoside, but there were consistent trends that will be discussed below.

Malvidin glucoside was the dominant anthocyanin. Molecular ions and their fragments obtained by MS for each anthocyanins are listed in Table 3. Total anthocyanins' HPLC values were similar to TACY values (two values were significantly correlated; 0.975, $p \leqslant 0.05$), as established by previous work (Lee & Finn, 2007).

All five individual anthocyanins tended to be lower in the infected samples, compared to the healthy samples in a given pair, and were significantly lower in VY2a-infected versus uninfected samples. The same trends were observed in VY1 and VY2b samples, but only peonidin glucoside was significantly lower in these infected vines, compared to healthy vines. These results confirm the reduction in colour observed in GLRaV-3 infected 'Nebbiolo' berries, as determined by HPLC (Guidoni et al., 1997). Krake (1993) reported a decrease in colour of 'Emperor' grapes that were infected, compared to healthy vines, but this report was based on visual assessment. Anthocyanin levels within the same vineyard block were impacted negatively when infected by GLRaV-3. Healthy berries from the two rootstock/scion combinations from VY2 were significantly different in cyaniding glucoside content. Total anthocyanins were not significantly different between the healthy samples from VY2, but VY2b berries were slightly higher in anthocyanins than those of VY2a. Anthocyanin composition differences due to virus status was easier to observe when the proportion of the individual anthocyanin values (individual anthocyanin/total anthocyanin $\times$ 100, which could be easily calculated from values reported in Table 2) were compared. All GLRaV-positive samples had a greater proportion of malvidin glucoside and a lower proportion of delphinidin glucoside, compared to their healthy counterparts, which was the reverse of what has been observed with 'Nebbolio' grapes by Guidoni et al. (1997). This observed difference could be due to the grape cultivar, virus type, and the fact that Guidoni et al. (1997) compared infected mother vines with heat-treated (i.e., GLRaV-3 and grapevine virus A eliminated by heat treatment) progeny vines. GLRaV-3 may influence anthocyanin profiles differently depending on cultivar, but among 'Pinot noir' berries examined in this study the trend was similar for GLRaV-2 and GLRaV-3 infections.

Fig. 1 shows the anthocyanin content of sample pairs, collected approximately weekly, beginning two or more weeks before commercial harvest. VY1-2 (sampled 26/9/2005) and VY1-3 (sampled 03/10/2005) were the only pairs that were significantly different. In general, all samples (with the exception of VY2b-3, collected 15/9/2006) from GLRaV-positive vines had lower anthocyanin levels, when compared to their GLRaV-negative counterparts. Anthocyanin appeared to have accumulated earlier in GLRaV-negative vines, which Guidoni et al. (1997) noted as well. It is unknown if GLRaV-positive clusters are commonly left to hang longer in the field than their healthy counterparts, or if infected berries can reach the same anthocyanin levels as healthy berries. But, it would not be economical for a commercial operation to harvest the same vineyard multiple times due to virus symptoms. Variation in total anthocyanins from different sampling periods, or seasons, was also observed by Guidoni et al. (1997) and Mazza et al. (1999). GLRaV-positive samples from all collection dates had a larger proportion of malvidin glucoside compared to GLRaV-negative ones, except for samples taken during the earliest dates from VY2a and VY2b (i.e., in VY2a-3, VY2b-1, and VY2b-3 pairs this trend was reversed). It is unknown how GLRaV infection influences anthocyanin metabolism and accumulation from veraison to harvest, and would be an interesting topic but was not within the scope of this study.

3.3. Polyphenolics by HPLC

Fraction **a** (skins and pulp) contained 13 individual polyphenolics (Table 3); four phenolic acids (protocatechuic acid, caftaric acid, coutaric acid, and fertaric acid), five flavanols (gallocatechin, procyanidin 1, catechin, procyanidin 2, and epicatechin), and four flavonol glycosides (quercetin galactoside, quercetin glucuronide, kaempferol glucoside, and isorhamnetin glucoside). Trace amounts of gallocatechin (peak FA1) were found in each fraction **a** sample, but were not included in the quantification. Caftaric acid was the main phenolic acid, catechin was the main flavanol, and quercetin glucuronide was the main flavonol glycoside in all 'Pinot noir' berries, which confirms Price et al. (1995) findings. Fraction **b** (seeds)

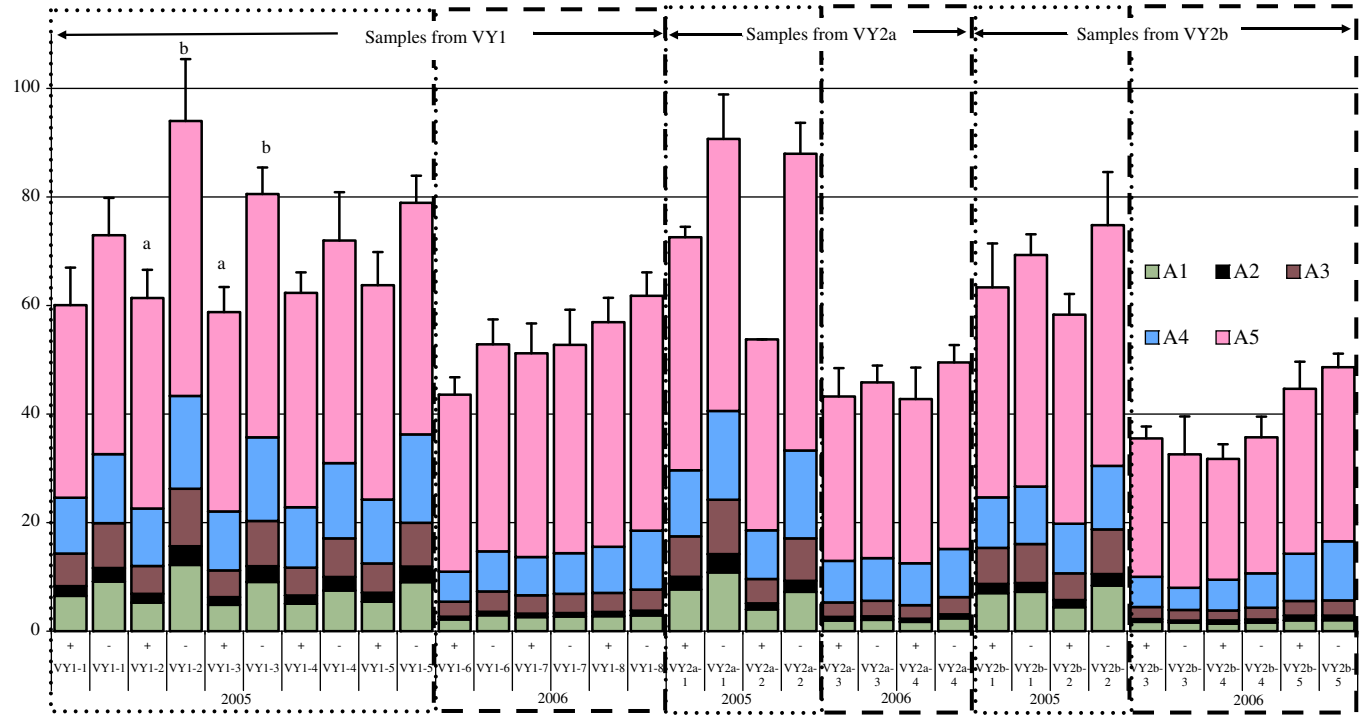

Fig. 1. Anthocyanin accumulation during the sampling periods from VY1, VY2a, and VY2b. The anthocyanin codes (A1–A5) are listed in Table 3. '+' Indicates GLRaV-positive and '−' indicates GLRaV-negative by RT-PCR. The corresponding sampling dates (e.g. VY1-1 sampled 9/19/2005) are listed in Table 1. Values were obtained by HPLC as mg/100 g of berries. Different lower case letter indicates significant different at $p \leqslant 0.05$ for the pair of samples (infected versus healthy). Error bars indicate standard errors.

contained five flavanol monomers (catechin was the major) and dimers (listed in Table 3).

GLRaV status did not statistically alter individual or total polyphenolics in samples from VY1 or VY2b (Table 3). Protocatechuic

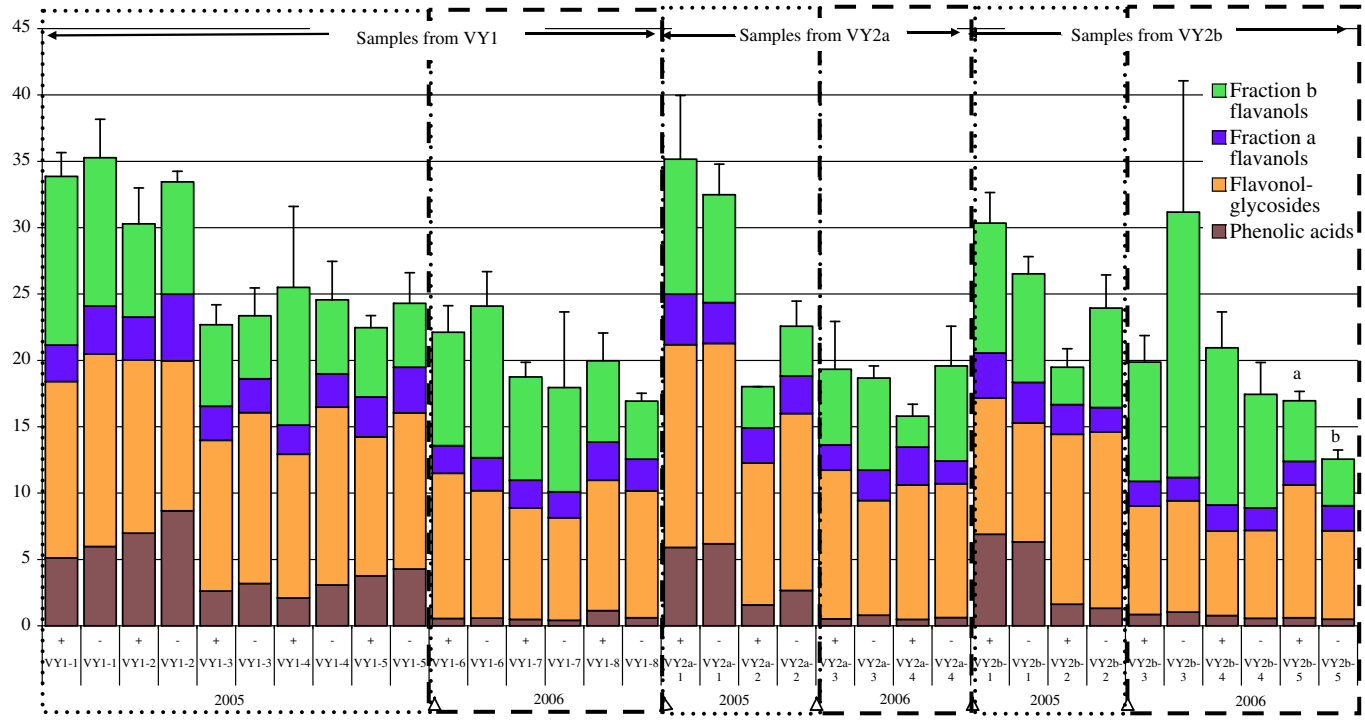

Fig. 2. Polyphenolic accumulation during the sampling periods from VY1, VY2a, and VY2b. '+' Indicates GLRaV-positive and '−' indicates GLRaV-negative by RT-PCR. The corresponding sampling dates (e.g. VY1-1 sampled 19/9/2005) are listed in Table 1. Polyphenolic values were grouped into the three phenolic classes. Phenolic acids consisted of FA2, FA3, FA5, and FA7. Flavonol glycosides were peaks FA10–FA13. Flavanols were grouped as fraction **a** (FA4, FA6, FA8, and FA9) and fraction **b** (FB1–FB5). Peak codes are listed in Table 3. Sampling date codes are in Table 1. Values were obtained by HPLC as mg/100 g of berries. Different lower case letters indicates significant different at $p \leqslant 0.05$ for the pair of samples (infected versus healthy). Error bars indicate standard errors.

acid and quercetin galactoside were significantly lower, though procyanidin 2 (FA8) was significantly higher, in GLRaV-3 positive samples from VY2a. Overall, total skin polyphenolic content of whole berries was lower in infected vines from VY1 and VY2a, but not from VY2b, again implying that rootstock/scion combination responded in a different way to GLRaV infection. Seed polyphenolics (fraction **b**) were numerically, but not significantly, lower in both infected samples from VY2. While not significant, total polyphenolics of whole berries were lower in healthy samples from VY1 and VY2b, but not VY2a. GLRaV-2 infected samples from VY1 had an increase in the proportion of flavanols and decreased flavonol glycosides, compared to their virus-negative counterparts at harvest. GLRaV-3 infection decreased the proportion of flavanols and increased flavonol glycosides at harvest in both rootstock/scion combinations. GLRaV-3 infection influenced phenolic acids differently in grapes from VY2, it decreased in VY2a-infected samples and increased in VY2b infected samples, compared to their healthy counterparts.

The polyphenolics from all sample collection dates (two or more weeks before commercial harvest) were combined into the three phenolic classes, paired as infected (+) and virus-negative (−), and presented in Fig. 2. Absolute flavanol concentrations and their proportional concentration in seeds (fraction **b**) slightly decreased as the sampling dates got closer to harvest, which has also been reported in seeds of 'Shiraz' and 'Cabernet Sauvignon' berries (Kennedy, Matthews, & Waterhouse, 2000; Kennedy et al., 2000). The percent of fraction **b** flavanols was higher in infected vines from VY1 and VY2a, but the opposite occurred in VY2b. Unlike anthocyanins, no clear trends regarding the polyphenolics of infected versus healthy vines were observed (Fig. 2). Also, the change in proportion of these different phenolic classes altered depending on the growing season. Infected 'Nebbiolo' vines have shown higher levels of flavonol glycosides and epicatechin, but lower levels of phenolic acids (Guidoni et al., 1997). Trends were not noticed in the polyphenolics of infected versus healthy vines leading up to harvest in 'Nebbiolo' grapes either (Guidoni et al., 1997).

It is possible that commercial vineyard practices (cluster thinning, pruning, etc.) to control vine vigour may have been biased by visual symptoms of viruses, and could have influenced our findings, and contributed toward numerous analyses that were not significantly different. Our future work will eliminate such unknowns by conducting the investigation in a controlled research plot with known time of infection, rootstock/scion combinations, and vineyard management practices.

4. Conclusion

To the best of our knowledge, this is the first report of the impact of GLRaV on phenolic compounds in 'Pinot noir' berries from commercial vineyards in Oregon. This preliminary work provides insight into the variation of phenolics due to GLRaV and grapevine interactions. Differences in response to virus infection within the same cultivar 'Pinot noir' with different rootstock/scion combinations were observed. *Vitis riparia* rootstock/'Pinot noir' clone 114 scion combination appeared to be more sensitive to GLRaV infection than the other two rootstock/scion combinations. Berries from the same vineyard block (VY2) with different rootstock/scion combinations had different trends (seed weight, pH, TA, TP, TT, and total polyphenolics in healthy versus infected vines). This indicates that rootstock/scion combinations are important in determining responses to GLRaV-3 infection. The decrease of berry anthocyanin from infected vines emphasises the importance of virus-free grapevine stock (a trend in all three rootstock/scion combinations). Future work is needed to better understand the relationship between GLRaV and phenolics, in a controlled environment with known rootstocks, scions, vine ages, and period of infections.

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