

# Influence of grapevine leafroll associated viruses (GLRaV-2 and -3) on the fruit composition of Oregon *Vitis vinifera* L. cv. Pinot noir: Free amino acids, sugars, and organic acids

Jungmin Lee<sup>a,\*</sup>, Karen E. Keller<sup>b</sup>, Christopher Rennaker<sup>a</sup>, Robert R. Martin<sup>b</sup>

<sup>a</sup> United States Department of Agriculture, Agricultural Research Service, PWA, Horticultural Crops Research Unit Worksite, 29603 U of I Ln., Parma, ID 83660, USA

<sup>b</sup> United States Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Unit, Corvallis, OR 97330, USA

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## ABSTRACT

Individual free amino acids, yeast assimilable amino acid (YAN) content, ammonia, organic acids, and simple sugars of berries from vines infected with GLRaV-2 or -3 were compared with paired vines free of these viruses. Samples were taken from two commercial vineyards during two growing seasons (2005 and 2006), with three different rootstock/scion combinations. Vines infected with GLRaV-2 did not differ significantly from their healthy counterparts in individual free amino acids, ammonia, or YAN content. Vines infected with GLRaV-3 were significantly lower in valine and methionine from *Vitis riparia* rootstock/'Pinot noir' clone 114 (VY2a) samples, and lower in glutamic acid from self-rooted/'Pinot noir' clone Pommard (VY2b) samples, compared to samples from their healthy counterparts. Samples from VY2b (self-rooted/'Pinot noir' clone Pommard) infected vines had significantly lower levels of malic acid and total organic acids compared to samples from their healthy counterparts. There were no significant differences between healthy and infected vines from all three rootstock/scion pairs in ammonia or free amino acids in samples taken during the weeks before ripening and at commercial harvest. This is the first study to report the influence of GLRaV-2 and -3 on 'Pinot noir' berries nitrogen (N) compounds significant to fermentation. Individual free amino acids may be inferior to phenolic compounds as indicators of GLRaV infection status.

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## 1. Introduction

Nitrogen-containing compounds found in grapes have been reported to vary, depending on cultivar, vine nutrition, vineyard management, soil type, soil moisture content, vine virus status, grape maturity and growing season (Bell & Henschke, 2005 references therein; Conde et al., 2007; Kliewer & Lider, 1976; Stines et al., 2000; Ueno, Kinoshita, Togawa, & Iri, 1985). Vineyard management practices and environmental factors that influence the amount of nitrogen (N) found in grapes and wine have been reported (Bell & Henschke, 2005).

Amino acids are primary metabolites that are important to grapevines' survival, but are also valuable nutrients for the yeasts and bacteria responsible for alcohol and malolactic fermentations (Bell & Henschke, 2005). Ammonia and certain free amino acids, which are primary amines, impact fermentation rate and the completion of fermentation, and these compounds are known as yeast

assimilable nitrogen (YAN). Grape cultivar, must pH, yeast strain, and other factors have been reported to influence the utilisation of the available must N during fermentation (Bell & Henschke, 2005; Garde-Cerdan & Ancin-Azpilicueta, 2008; Monteiro & Bisson, 1991; Taillandier, Portugal, Fuster, & Strehaiano, 2007). Low grape YAN can lead to stuck or sluggish fermentation and potentially produce undesirable compounds in the resulting wine, such as sulfur and thiol-containing compounds like hydrogen sulfide, or fusel alcohols (Bell & Henschke, 2005; Beltran, Esteve-Zaraoso, Rozes, Mas, & Guillamon, 2005; Vilanova et al., 2007). Fermentation conditions (must temperature, oxygen level, timing of N addition, form of N addition, etc.) and juice components such as sugar and vitamin levels alter the yeast fermentation kinetics for different forms of available N (Arias-Gil, Garde-Cerdan, & Ancin-Azpilicueta, 2007; Beltran et al., 2005; Taillandier et al., 2007). It is still unknown if YAN compounds derived from grape are superior to N supplements from diammonium phosphate (DAP) for a healthy fermentation of N-deficient must into fine wine. Some amino acids are precursors to particular volatile compounds and recent studies have demonstrated that the addition of free amino acids increased a number of wine volatiles (Garde-Cerdan & Ancin-Azpilicueta, 2008; Hernandez-Orte, Cacho, & Ferreira, 2002).

\* Corresponding author. Tel.: +1 208 722 6701x282; fax: +1 208 722 8166.

E-mail addresses: [jlee@uidaho.edu](mailto:jlee@uidaho.edu), [jungmin.lee@ars.usda.gov](mailto:jungmin.lee@ars.usda.gov) (J. Lee), [karen.keller@ars.usda.gov](mailto:karen.keller@ars.usda.gov) (K.E. Keller), [chris.rennaker@ars.usda.gov](mailto:chris.rennaker@ars.usda.gov) (C. Rennaker), [bob.martin@ars.usda.gov](mailto:bob.martin@ars.usda.gov) (R.R. Martin).

'Pinot noir' grapes and wines are major economic contributors to the state of Oregon, where 'Pinot noir' is the dominant cultivar grown, and made up more than 50% of the 38,000 tons of wine grapes produced in 2007. 'Pinot noir' production in Oregon is followed, in order of tons produced, by 'Pinot gris', 'Chardonnay', 'Riesling', and 'Cabernet Sauvignon' (National Agricultural Statistics Service, 2008a).

The presence of grapevine leafroll associated viruses (GLRaV) has been reported in Oregon vineyards (Martin, Eastwell, Wagner, Lamprecht, & Tzanetakis, 2005). GLRaV status has the potential to alter berry composition, beyond changes in their phenolic profiles (Lee & Martin, 2009; Singh Brar, Singh, Swinny, & Cameron, 2008), and N containing compounds (Kliwer & Lider, 1976; Ueno et al., 1985). To the best of our knowledge, there are only reports on the impact of GLRaVs on free amino acids of 'Burger' ('Burger' scion grafted onto 'Dogridge' rootstock), 'Zenkoi', and 'Koshu' grapes (Kliwer & Lider, 1976; Ueno et al., 1985), and none on the cultivar 'Pinot noir'.

GLRaVs are phloem-limited viruses that can result in reduced net leaf photosynthesis, decreasing % soluble solids and reducing vine productivity. Because of this, there is interest in exploring how GLRaV infections alter berry sugars and organic acids that contribute greatly to the taste and final alcohol content of the wine (Bertamini, Muthuchelian, & Nedunchezian, 2004; Cabaleiro, Segura, & Garcia-Berrios, 1999; Christov et al., 2007; Guidoni, Mannini, Ferrandino, Argamante, & Di Stefano, 1997; Kliwer & Lider, 1976; Lee & Martin, 2009; Ueno et al., 1985).

It is well established that GLRaVs impact grape differently depending on cultivar, rootstock, scion, vine age, and causal virus (Cabaleiro et al., 1999; Golino, 1993; Guidoni et al., 1997; Kliwer & Lider, 1976; Kovacs, Hanami, Fortenberry, & Kaps, 2001; Krake, 1993; Lee & Martin, 2009; Singh Brar et al., 2008; Wolpert & Vilas, 1992). A recent report conducted in our laboratories (Lee & Martin, 2009) demonstrated that GLRaVs imparted a significant decrease in individual and total anthocyanins in 'Pinot noir' berries from *Vitis riparia* rootstock/'Pinot noir' clone 114 scion, with other minor differences in non-anthocyanin phenolics, but no significant difference in anthocyanins from GLRaV were observed in the other two rootstock/scion combinations examined (unknown rootstock/'Chardonnay' interstock/'Pinot noir' scion [clone unknown] and self-rooted/'Pinot noir' clone Pommard). It is important to understand how GLRaVs impact compounds beyond phenolics (i.e., N-containing compounds) that influence wine grape quality and healthy fermentations.

The objective of this study was to compare the compounds important for a healthy fermentation (i.e., simple sugars, organic acids, ammonia, and free amino acids) of 'Pinot noir' berries from vines infected by GLRaV-2 or -3 with berries from vines free of these viruses, to better understand how GLRaVs impact 'Pinot noir' grape quality.

## 2. Materials and methods

### 2.1. Plant material

Details of the samples, vineyards, vines, collection dates, and harvest dates were presented in Lee and Martin (2009), but for convenience, the information has been reproduced with modifications in Table 1, with the same abbreviations being used for this paper. All grapes were obtained from commercial fields. Briefly, four clusters were randomly taken from each presumptively identified healthy or infected vine (at each reported collection date; Table 1) and stored at  $-23^{\circ}\text{C}$ , until virus status was determined for each vine by RT-PCR in the laboratory. All clusters from a vine were grouped for chemical analysis. Vineyards one (VY1; one rootstock/

scion combination) and two (VY2a and VY2b representing the two different rootstock/scion combination sampled from vineyard 2) were approximately 40 km apart. Both vineyards were located in Oregon's Willamette Valley (USA).

### 2.2. Reagents, chemicals, and standards

All chemicals for ammonia, organic acids, simple sugars, and free amino acid standards analyses were obtained from Sigma Chemical Co. (St. Louis, MO). Chemicals for the in-line derivatisation prior to HPLC injection were purchased from Agilent Technologies Inc. (Santa Clara, CA). All solvents and chemicals for this investigation were analytical and high performance liquid chromatography (HPLC) grade.

### 2.3. Virus detection

Virus status was determined as reported previously (Martin et al., 2005). Alterations to the detection procedure were described in Lee and Martin (2009).

### 2.4. Extraction and sample preparation

Berries were excised from the clusters (four clusters per vine from 5 to 11 vines depending on virus status; Table 2). Whole berries were puréed with a hand blender for 3 min (which macerated skin, pulp, and seeds), then centrifuged for 10 min at 4000 rpm. The resulting supernatants were then filtered with disposable 25 mm GD/X syringe filters (Whatman Inc., Florham Park, NJ) prior to organic acids, simple sugars, and ammonia determination. Disposable Millex-FH syringe filters (Millipore, Bedford, MA) were used for supernatants prior to free amino acid determination.

### 2.5. HPLC conditions for organic acids, sugars, and free amino acids analyses

An Agilent HP1100 system (Agilent Technologies Inc.) equipped with a diode array detector (DAD) connected to refractive index detector (RID), and a Rezex ROA-organic acid H+ (300 mm  $\times$  7.8 mm, 8  $\mu\text{m}$ ; Phenomenex, Torrance, CA) column were used for organic acid and sugar analyses. A ratio of 80% diluted sulfuric acid (2.5 mM sulfuric acid solution) to 20% acetonitrile was used as the mobile phase (m.p.) at a flow rate of 0.5 ml/min under isocratic conditions. The analytical column compartment was maintained at  $55^{\circ}\text{C}$  during the 15 min analysis. Organic acids were detected at 210 nm with a DAD. RID was used for simple sugar identification and quantification. Tartaric acid, malic acid, fumaric acid, glucose, and fructose external standards were used. The malic acid standard was corrected for the fumaric acid contamination that occurs in all malic acid standards. Sugar and organic acid values were expressed as mg/100 ml.

HPLC/DAD was used for individual free amino acid analysis. Zorbax Eclipse AAA analytical (150 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ , Agilent Technologies Inc.) and guard (12.5 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ; Agilent Technologies Inc.) columns were used for free amino acid determination. Inline-derivatisation by *o*-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) was performed by the autosampler immediately prior to injection, as described in detail by Henderson, Ricker, Bidlingmeyer, and Woodward (2006). Mobile phase A was 40 mM sodium phosphate (adjusted to pH 7.8 with 10 N sodium hydroxide solution). Mobile phase B was a mixture of acetonitrile: methanol: water (45:45:10, v/v/v). HPLC mobile phase conditions were extended from the gradient program described by Schuster (1988) and Henderson et al. (2006) to suit our HPLC and column set-up. Three min extension of the gradient (m.p. B was 57–81% and the rest of the m.p. was made up by m.p. A

**Table 1**

Description of samples taken from two commercial vineyards in Oregon (Lee &amp; Martin, 2009).

	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)	<i>V. riparia</i> 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 after testing	GLRaV-2 positive or negative; RSPaV positive	GLRaV-3 positive or negative; RSPaV positive	GLRaV-3 positive or negative; RSPaV positive
Sampling dates for both seasons. Codes used in Figs. 1–3. Dates in bold were final commercial harvest dates	VY1-1: 9/19/2005  VY1-2: 9/26/2005 VY1-3: 10/3/2005 VY1-4: 10/11/2005 <b>VY1-5: 10/17/2005</b> VY1-6: 9/15/2006 VY1-7: 9/22/2006 <b>VY1-8: 9/28/2006</b>	VY2a-1: 9/28/2005  <b>VY2a-2: 10/3/2005</b> VY2a-3: 9/15/2006 <b>VY2a-4: 9/22/2006</b>	VY2b-1: 9/28/2005  <b>VY2b-2: 10/3/2005</b> VY2b-3: 9/15/2006 VY2b-4: 9/22/2006 <b>VY2b-5: 9/28/2006</b>

**Table 2**

Virus status, simple sugars, organic acids, ammonia, individual free amino acids, total free amino acids (FAN) and yeast assimilable nitrogen (YAN) at harvest from all locations are listed. All sampled vines tested positive for RSPaV. Different lower case letters indicate significant differences ( $p \leq 0.05$ ) within the pair of samples (e.g., samples from GLRaV-2 positive versus negative vines from vineyard 1). Different upper case letters indicate significant differences ( $p \leq 0.05$ ) among the three healthy 'Pinot noir' samples (comparison among VY1, VY2a, and VY2b). No lower or upper case letters after values indicate no significant difference. Values in parenthesis are standard errors.

Sample code from the two vineyards	VY1	VY2a	VY2b
Harvest dates	10/17/2005 and 9/28/2006	10/03/2005 and 9/22/2006	10/03/2005 and 9/28/2006
Virus status	GLRaV-2 positive	GLRaV-3 positive	GLRaV-3 positive
Number of vines corresponding to the GLRaV results	7	5	6
Total simple sugars (g/100 ml)			
Glucose	24.4 (1.6)	25.0 (0.7)	25.6 (0.9)
Fructose	11.9 (0.3)	12.4 (0.4)	12.5 (0.5)
Total organic acids (g/100 ml)			
Tartaric acid	12.5 (0.3)	12.6 (0.3)	13.1 (0.4)
Malic acid	0.81 (0.03)	0.78 (0.02) B	0.63 (0.03) a
Ammonia in juice (mg N/l)	0.43 (0.02)	0.40 (0.02)	0.45 (0.02)
Total free amino acids (mg N/l) = FAN	0.38 (0.03)	0.30 (0.02)	0.25 (0.02) a
1 Aspartic acid (ASP)	43.3 (3.2)	20.8 (1.3)	19.0 (3.9)
2 Glutamic acid (GLU)	421.4 (44.4)	177.6 (13.3) A	150.4 (29.2)
3 Asparagine (ASN)	2.6 (0.4)	3.0 (0.6)	3.3 (0.3)
4 Serine (SER)	4.5 (0.6)	1.5 (0.1)	1.5 (0.3) a
5 Glutamine (GLN)	1.5 (0.8)	1.0 (0.1)	0.7 (0.1)
6 Histidine (HIS)	11.4 (2.5)	7.6 (0.5)	8.2 (0.5) B
7 Glycine (GLY)	19.9 (5.1)	12.2 (0.9)	14.3 (1.0) A
8 Threonine (THR)	13.8 (1.8)	7.3 (0.6)	7.4 (0.6) A
9 Citrulline (CIT)	1.2 (0.1)	1.1 (0.2)	0.9 (0.1)
10 Arginine (ARG)	27.0 (1.9)	14.4 (1.2)	14.0 (2.4)
11 Alanine (ALA)	2.8 (0.5)	1.1 (0.1)	0.9 (0.2)
12 Tyrosine (TYR)	231.7 (28.6)	73.6 (8.2)	62.0 (16.9)
13 Valine (VAL)	26.0 (2.3)	15.5 (1.3)	10.8 (0.8)
14 Methionine (MET)	0.6 (0.1)	0.3 (0.0)	0.2 (0.0)
15 Tryptophan (TRP)	2.9 (0.4)	2.7 (1.6) a	6.6 (1.0) b B
16 Phenylalanine (PHE)	0.4 (0.1)	0.3 (0.2) a	1.5 (0.3) b B
17 Isoleucine (ILE)	4.1 (0.6)	2.0 (0.2)	2.7 (0.2) A
18 Leucine (LEU)	4.8 (1.0)	2.0 (0.3)	2.8 (0.4) A
19 Lysine (LYS)	4.6 (1.0)	1.7 (0.3)	2.6 (0.4) A
20 Hydroxyproline (HYP)	7.3 (1.4)	3.2 (0.4)	3.7 (0.7) A
21 Proline (PRO)	1.4 (0.1)	0.8 (0.1)	1.1 (0.4)
YAN (mg N/l)	31.3 (3.9)	12.3 (1.7)	12.5 (1.6) A
	21.8 (2.3)	13.8 (2.8)	16.6 (1.8) A
	411.7 (44.7)	172.3 (12.8)	180.5 (12.8) A
	392.1 (30.4) B		148.7 (29.0)
			153.6 (19.7) A

from 43% to 19%) was included at the end of the analytical conditions described by Henderson et al. (2006). OPA-derivatised amino acids were monitored at 338 nm and FMOC-derivatised amino acids were monitored at 262 nm. Purchased standards of each individual amino acid (Sigma Chemical Co.) were used for identification and quantification (external standard method). Two internal standards, norvaline for OPA-derivatised amino acids and sarcosine for FMOC-derivatised amino acids, were used. Individual free amino acid values were expressed as mg N/l.

## 2.6. Ammonia concentration and calculation of YAN values

Ammonia was quantified by an enzymatic assay (Sigma ammonia assay kit; Sigma Chemical Co.) using a spectrophotometer (SpectraMax M2 microplate reader; Molecular Devices Corp., Sunnyvale, CA). Manufacturer instructions were followed. One-centimeter path length disposable semi-micro cuvettes (Fisherbrand, Thermo Fisher Scientific Inc., Waltham, MA) were used for ammonia measurements. YAN content was calculated by combining the

ammonia concentration found in the berries with primary free amino acids. Primary free amino acid content was determined by excluding hydroxyproline and proline contents from total free amino acids found in the berry samples. Ammonia and YAN were expressed as mg N/l.

### 2.7. 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 the pair of samples from infected and healthy vines with the same rootstock/scion combination ( $\alpha = 0.05$ ). Correlation between organic acid values determined by HPLC and titratable acidity (values from Lee and Martin (2009)), sugars determined by HPLC and % soluble solids (values from Lee and Martin (2009)), and between ammonia and primary amino acid values were calculated ( $\alpha = 0.05$ ). Differences among the three different rootstock/scion the values were compared using Fisher's LSD (least significant difference;  $\alpha = 0.05$ ).

## 3. Results and discussion

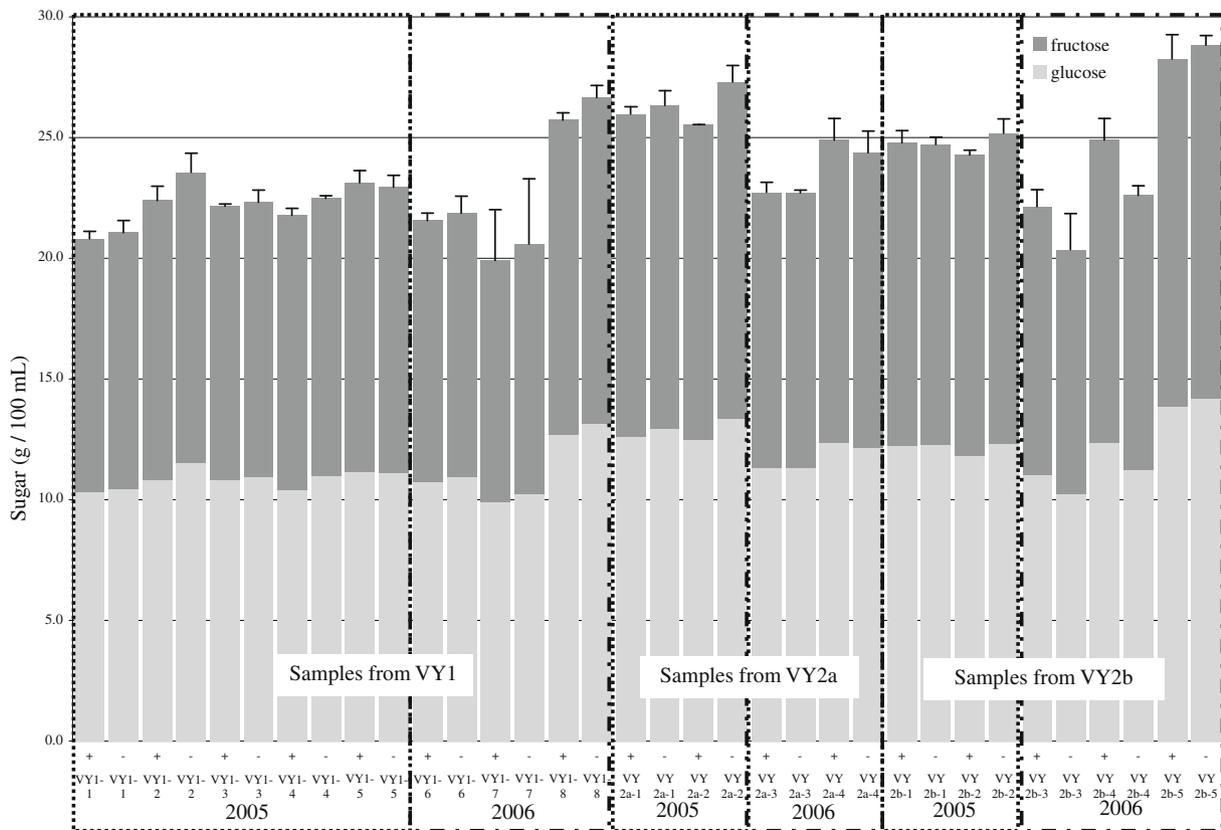
Again, details of the vineyard location and vine virus status were reported in the first part of this project (Lee & Martin, 2009). Briefly, all vines despite their GLRaV status in both vineyard locations tested positive for grapevine Rupestris stem pitting-associated virus (RSPaV). GLRaV-infected vines from VY1 had co-infection of RSPaV and GLRaV-2. VY2a and VY2b GLRaV-positive vines were co-infected with RSPaV and GLRaV-3. Vineyard and virus data are summarised in Table 1. The observed results in this study were

assumed to be due to GLRaV status despite the co-infection with RSPaV. RSPaV has been reported as having little or no significant impact on the yield components of titratable acidity, and pH in five grape cultivars ('Kerner', 'Michurinetz', 'Okanagan Riesling', 'Madeleine Sylvaner', and 'Ortega'; Reynolds, Lanterman, & Wardle, 1997). Additional details of the vineyards and vines can be found in Lee and Martin (2009). There was no significant interaction between growing season and vine virus status, so the data of two seasons are pooled and presented in Table 2.

### 3.1. Simple sugars and organic acids

Two simple sugars, glucose and fructose, were found in all samples; glucose and fructose as reported by others (Diakou et al., 1997; Kliewer, 1967; Kliewer, Lider, & Schultz, 1967). In general, sugar content in all samples steadily increased during the sampling period up to harvest (Fig. 1). The ratio of glucose to fructose ranged from 0.91 to 1.02. There were no significant differences between fruit from infected and healthy vines in their glucose and fructose contents throughout the sampling periods (Fig. 1; Table 2). GLRaV infection status did not alter sugar composition or content.

Tartaric acid and malic acid were the two main organic acids identified in 'Pinot noir', which have been reported by others (Diakou et al., 1997; Kliewer et al., 1967). When both seasons were combined, there were no differences in tartaric and malic acids at commercial harvest between healthy and infected vines from VY1 or VY2a (Table 2). Samples from healthy vines in VY2b had significantly higher levels of malic acid and total organic acids compared to those from GLRaV-3 infected vines when both growing season values were compared (berry sizes were not significantly different; Lee & Martin, 2009). In 2006, samples from VY2a vines infected



**Fig. 1.** Simple sugars during the sampling periods from VY1, VY2a, and VY2b. The '+' indicates GLRaV-positive and '-' indicates GLRaV-negative by RT-PCR. VY1-5, VY1-8, VY2a-2, VY2a-4, VY2b-2, and VY2b-5 indicate commercial harvest. The corresponding sampling dates (e.g., VY1-1 sampled 9/19/2005) are listed in Table 1. Sugar values were obtained by HPLC as g/100 ml. No pairs were significantly different at  $p \leq 0.05$  (infected versus healthy). Error bars indicate standard errors.

with GLRaV-3 were significantly higher in malic acid at harvest compared to healthy vines (Fig. 2), which was not observed in 2005 and did not significantly alter total organic acid content.

Total sugar and total organic acid values obtained in this study were highly correlated ( $r$  values of 0.964 and 0.605,  $p \leq 0.05$ ) to °Brix and titratable acidity values, respectively (Lee & Martin, 2009).

GLRaV (type not reported) eliminated (negative) 'Zenkoji' and 'Koshu' grapes had higher levels of glucose and fructose (Ueno et al., 1985) compared to their infected cohorts. Guidoni et al. (1997) reported no significant difference in malic and tartaric acids in 'Nebbiolo' healthy vines versus vines infected with GLRaV-3. GLRaV (type not reported) positive 'Burger' grapes (Kliewer & Lider, 1976) were reported to have higher levels of titratable acidity, malic acid, and tartaric acid compared to healthy vines.

A comparison among the healthy vines from the three rootstock/scion combinations were made, and VY2b samples were significantly higher in glucose, fructose, and total sugar compared to VY1 samples (Table 2) at the time of harvest. Healthy VY1 samples were significantly higher in malic acid and total organic acids, although tartaric acid was not different among the three different rootstock/scion combinations.

### 3.2. Ammonia, FAN (free amino acids), and YAN of grapes

Ammonia content (main mineral N source) ranged from 19.0 to 49.2 mg N/l at harvest (contributed to 10–14% of grape YAN; Table 2). Grapes from VY1 had overall higher levels (more than double) of ammonia (Table 2 and Fig. 3) than VY2a and VY2b samples. There were no significant differences in ammonia content between

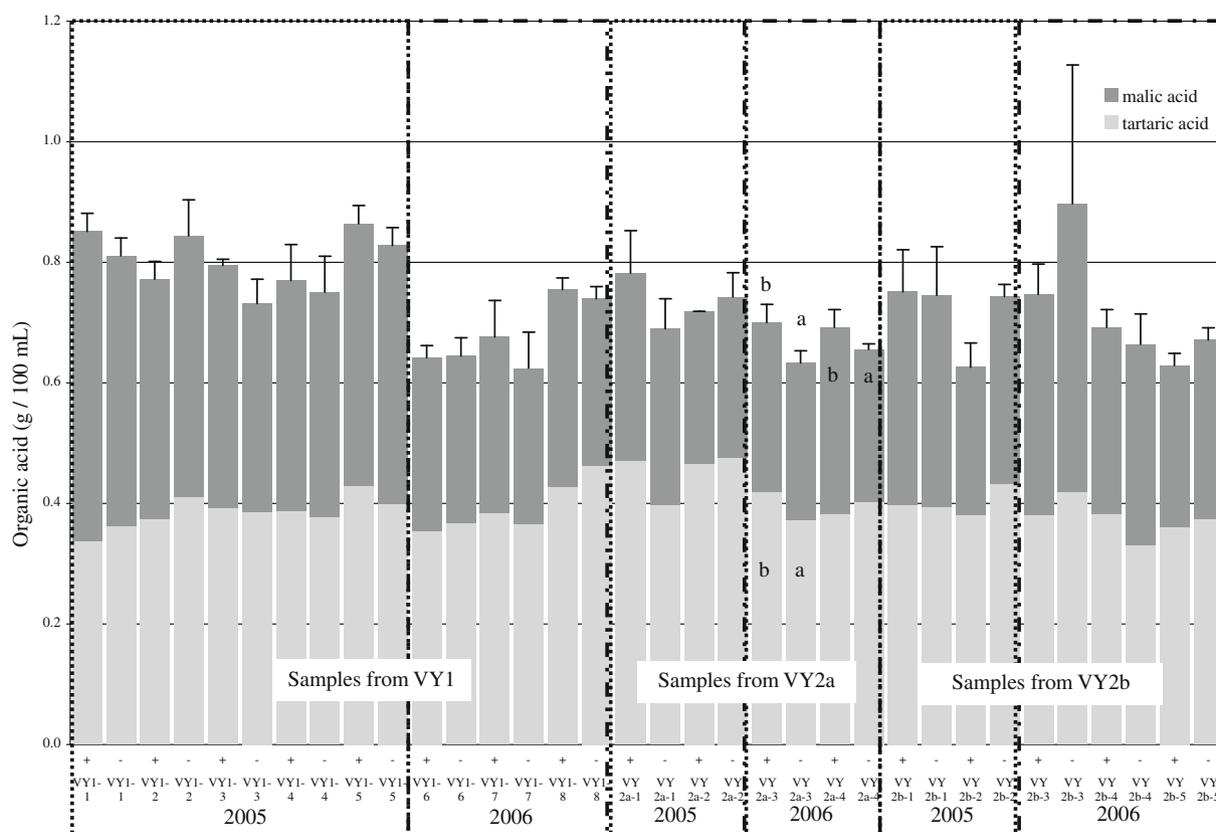
GLRaV-infected and healthy vines in the weeks prior to harvest or at commercial harvest in VY1, VY2a, and VY2b samples (Fig. 3).

Twenty-one free amino acids (Asp, Glu, Asn, Ser, Gln, His, Gly, Thr, Cit, Arg, Ala, Tyr, Val, Met, Trp, Phe, Ile, Leu, Lys, Hyp, and Pro; in the order of HPLC elution) were found in all samples, and are listed in Table 2. ARG was the main free amino acid, contributing to 30–62% of total free amino acids (FAN), in all 'Pinot noir' grapes, as reported by Huang and Ough (1991) and Stines et al. (2000).

Individual free amino acids, FAN, and YAN were not significantly different between the grapes from healthy and infected vines in VY1. Val and Met contents of VY2a healthy vines were significantly higher, compared to their infected counterparts. Only one amino acid, Glu from VY2b grapes, was significantly higher in grapes from healthy compared to infected vines. Overall, GLRaV infection did not impact N-containing compounds when the summation (YAN and FAN values) was compared in 'Pinot noir' grapes. The same statistical results were obtained when individual amino acid values were compared as mg of amino acids/l as well (data not shown). FAN and ammonia contents were not significantly different throughout the sampling period either (Fig. 3).

At harvest, all samples had an acceptable level of YAN (above 150 mg N/l) from the general guidelines of Bely, Sablayrolles, and Barre (1990), who recommends additional must nutrients (N supplementation in the must) if YAN is below 140 mg N/l for a healthy fermentation. Free amino acids were the major contributor to YAN values (86–90% of YAN was from free amino acids) in these samples at harvest as reported previously (Butzke, 1998).

Butzke (1998) performed a survey on commercial must of 'Pinot noir' grapes ( $n = 88$ ) and found ammonia of 92 mg N/l, primary



**Fig. 2.** Organic acids during the sampling periods from VY1, VY2a, and VY2b. The '+' indicates GLRaV-positive and '-' indicates GLRaV-negative by RT-PCR. VY1-5, VY1-8, VY2a-2, VY2a-4, VY2b-2, and VY2b-5 indicate commercial harvest. The corresponding sampling dates (e.g., VY1-1 sampled 9/19/2005) are listed in Table 1. Organic acids were obtained by HPLC as g/100 ml. Different lower case letter indicates significant differences at  $p \leq 0.05$  for the pair of samples (infected versus healthy). No lower case letter indicates no significant difference between healthy and infected vines. Error bars indicate standard errors.

amino acids 142 mg N/l, and YAN content at 236 mg N/l on average. Samples from VY2a and VY2b, despite virus status, were within what Butzke (1998) reported, but VY1 samples were well above these values.

Individual free amino acids were grouped into the following five groups: Ala-Val-Leu family (Ala, Val, and Leu), aromatic amino acid family (Phe, Tyr, His, and Trp), Asp family (Asp, Thr, Asn, Met, and Ile), Ser family (Ser and Gly), Glu family (Glu, Gln, Cit, Arg, Hyp, and Pro), and Lys, based on chemical similarities and biosynthesis pathways. There were no significant differences between samples from healthy and infected vines for all three rootstock/scion combinations. It appears that GLRaV did not alter the profile of amino acids in these rootstock/scion combinations when values were compared as grouped amino acid families.

At harvest, GLRaV-infected 'Burger' berries had significantly more ARG and less PRO than those from healthy vines (Kliwer & Lider, 1976) when compared to cluster-thinned vines. In 'Zenkoji' and 'Koshu' grapes, N and the majority of the free amino acids were lower in fruit from virus-eliminated vines (Ueno et al., 1985). These two previous publications and the work presented here demonstrate the varying impacts from GLRaV infection among grape cultivars, GLRaV type, etc.

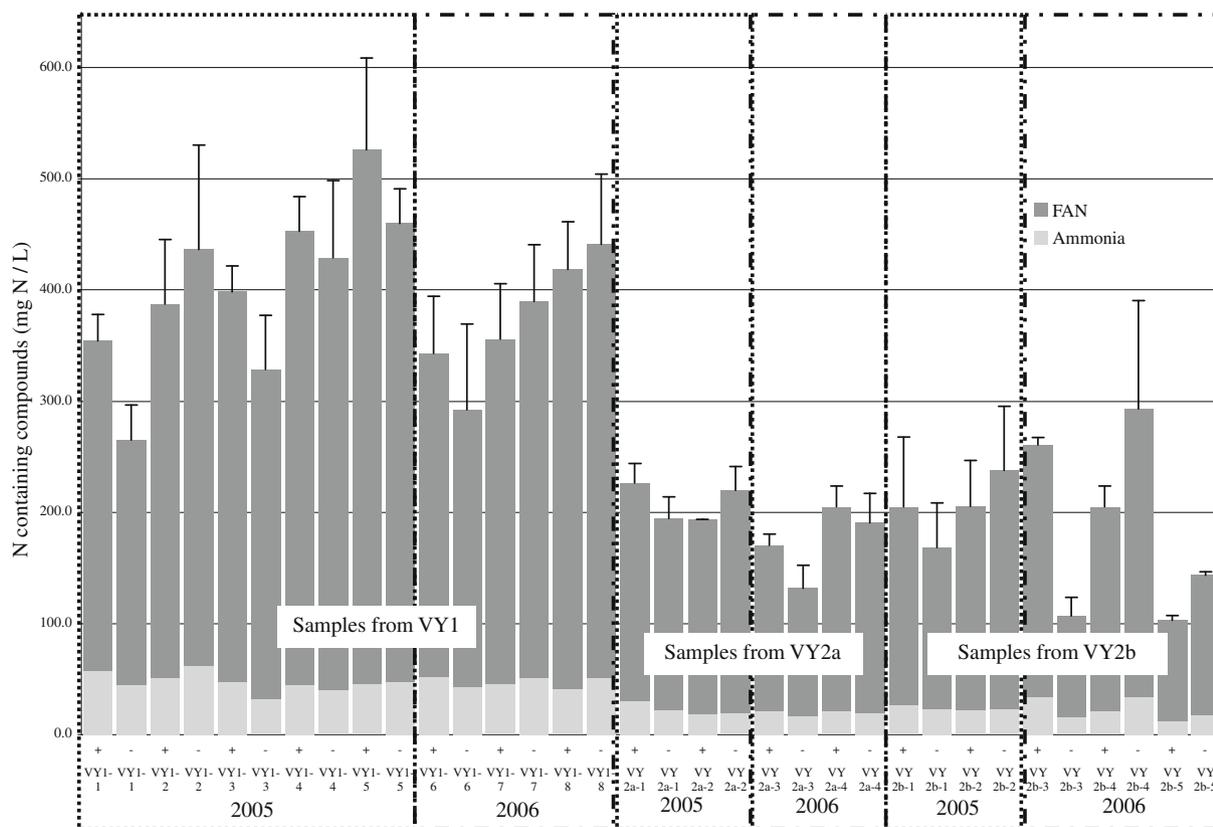
The individual amino acids, FAN, YAN, and ammonia were compared among the samples from the healthy vines from the three rootstock/scion combinations at harvest (Table 2). Berries from VY1 were significantly higher in individual free amino acids (except for Asp, Asn, Gly, Tyr, Val, Met, and Lys of VY2a samples or Asp, Gly, Val, Met, and Lys of VY2b samples), FAN, YAN, and ammonia than VY2a and VY2b samples. Berries from VY2a were signifi-

cantly higher in Val and Met, compared to samples from VY1 or VY2b. These observed variations might be due to rootstock/scion combinations, as was found in 'Chardonnay' (Treeby, Holzapfel, Walker, & Nicholas, 1998) and 'Shiraz' (Holzapfel & Treeby, 2007), or the disparity in vineyard management practices (i.e., levels, timing, and rate of N application in the vineyard; Bell & Henschke, 2005; Holzapfel & Treeby, 2007).

There was a significant correlation between primary amino acids and ammonia content in the juice samples ( $r = 0.86$  from all the juice samples and  $r = 0.924$  from time of commercial harvest  $p \leq 0.05$ ), which was not observed by Butzke (1998). He reported no correlation between primary amino acids and ammonia, and  $r$  of 0.29.

This preliminary work provides some insight into the minor differences in individual free amino acids, ammonia content, and YAN content of juice imparted by GLRaV infection. The lack of magnitude in the measured differences during this study might be due to decreased vine stress imposed by the Oregon vineyard practice of low crop load (average 2.8 tons per acre in Oregon for 2007; National Agricultural Statistics Service, 2008a) compared to other grape growing regions (e.g., on average 6–8 tons per acre in California for 2007; National Agricultural Statistics Service, 2008b).

Future work is needed to determine and clarify how vine age, virus infection period, rootstock/scion combination, and virus type influences the relationships among amino acids, sugars, and organic acids in an experimental vineyard setting, in order to eliminate the variability of commercial vineyard management. This future controlled study will allow us to understand the direct impact of GLRaVs.



**Fig. 3.** FAN and ammonia values during the sampling periods from VY1, VY2a, and VY2b. The '+' indicates GLRaV-positive and '-' indicates GLRaV-negative by RT-PCR. VY1-5, VY1-8, VY2a-2, VY2a-4, VY2b-2, and VY2b-5 indicate commercial harvest. The corresponding sampling dates (e.g., VY1-1 sampled 9/19/2005) are listed in Table 1. FAN were obtained by HPLC as mg of N/L. Ammonia (mg N/L) were determined by enzymatic assay. None of the paired samples were significantly different from each other. Error bars indicate standard errors.

#### 4. Conclusion

To the best of our knowledge, this is the first report upon the investigation of how GLRaVs impacted N-containing compounds, simple sugars, and organic acids in 'Pinot noir' berries from commercial vineyards in Oregon. In most comparisons, GLRaV did not significantly alter organic acids, simple sugars, ammonia, YAN, or FAN between fruit from healthy and infected vines. The exceptions were malic acid, total organic acid, Val + Met (in *V. riparia* rootstock/'Pinot noir' clone 114; VY2a), and Glu (in self-rooted/'Pinot noir' clone Pommard; VY2b) in GLRaV infected samples, compared to their GLRaV-negative cohorts. Among the healthy vines of the three rootstock/scion combinations, there was an apparent difference in the N-containing compounds, sugars, and acids, but this has been established for differing rootstock/scion combinations within a cultivar.

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