



Rootstock and vineyard floor management influence on 'Cabernet Sauvignon' grape yeast assimilable nitrogen (YAN)

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ABSTRACT

This is a study on the influence that two rootstocks (110R, high vigour; 420A, low vigour) and three vineyard floor management regimes (tilled resident vegetation – usual practise in California, and barley cover crops that were either mowed or tilled) had upon grape nitrogen-containing compounds (mainly ammonia and free amino acids recalculated as YAN), sugars, and organic acids in 'Cabernet Sauvignon' clone 8. A significant difference was observed for some of the free amino acids between rootstocks. In both sample preparation methods (juiced or chemically extracted), 110R rootstock grapes were significantly higher in SER, GLN, THR, ARG, VAL, ILE, LEU, and YAN than were 420A rootstock grapes. Differences in individual free amino acid profiles and concentrations were observed between the two sample preparations, which indicate that care should be taken when comparing values from dissimilar methods. No significant differences among vineyard floor treatments were detected, which suggests that mowing offers vineyard managers a sustainable practise, alternative to tilling, without negatively affecting grape nitrogen compounds, sugars, or organic acids.

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1. Introduction

Nitrogen compounds are important to grapevines and grapes, and in the winery they become measured constituents crucial to wine quality. These compounds (namely ammonia and primary free amino acids) are so essential to healthy alcoholic and malolactic fermentations in premium wine production (Bely, Sablayrolles, & Barre, 1990) that, before the addition of yeast, supplementation of the must with some form of nitrogen is a common winery practise (anonymous; personal communications). In grapes, these compounds can be influenced by cultivar, rootstock, vineyard practises, environmental conditions, and growing season (Bell & Henschke, 2005 and references therein). Grape growers and winemakers target moderate nitrogen values to prevent excessive shoot vigour and a reduction of yields for the vines, to ensure that musts avoid stuck/sluggish fermentations that can lead to the formation of undesirable haze and/or thiols in the resulting wines (i.e. juice) (Bell & Henschke, 2005; Beltran, Esteve-Zaraoso, Rozes, Mas, & Guillamon, 2005; Ugliano et al., 2009; Vilanova et al., 2007). Since

horticulture factors affect grape quality, it is important to understand how premium wine production begins in the vineyard.

Few published studies have examined the effect that cover crops and vineyard floor management practises have on grape quality-indicator compounds (Hostetler, Merwin, Brown, and Padilla-Zakour, 2007; Monteiro & Lopes, 2007; Sweet & Schreiner, 2010; Wheeler, Black, & Pickering, 2005). Wheeler et al. (2005) examined fruit maturity indices (pH, titratable acidity, and % of soluble solids) and ammonia content (enzymatic reaction with spectrophotometric measurement) in 'Cabernet Sauvignon' samples from grapevines which they considered highly vigorous. Monteiro and Lopes (2007) found minor differences due to cover crops and floor management in grape maturity indices, but reported changes mainly in titratable acidity, berry skin total phenolics, and berry skin anthocyanins. Hostetler, Merwin, Brown, and Padilla-Zakour (2007) noted no significant difference in measured fruit maturity indices (% of soluble solids, pH, titratable acidity, anthocyanins, and total phenolics) in four 'under the vine' floor managements. Sweet and Schreiner (2010) found no effects on several 'Pinot noir' grape maturity measurements (yield, cluster weights, % of soluble solids, pH, and titratable acidity) among seven between-the-row (i.e. alleyway) cover crop treatments; however, treatments did alter juice YAN (yeast assimilable nitrogen). Despite seasonal variations, they observed the peak YAN values from 'Pinot

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noir' grapes from a native grass mix treatment in 2004, and a clover mix treatment in 2005. They also recorded their lowest YAN values from a perennial grass and clover mix in 2004 and a native meadow mix in 2005.

Grapevine rootstock selections have been demonstrated to play a role in grape nitrogen profile and total content (Holzapfel & Treeby, 2007; Treeby, Holzapfel, Waler, & Nicholas, 1998). Though the mechanism by which this difference occurs in grape free amino acids is speculated to stem from specific nitrogen mobilisation patterns for individual rootstocks, it is still not completely understood (Holzapfel & Treeby, 2007; Treeby et al., 1998). While vineyard floor management practises alone, such as the type of cover crop and the timing of its planting, mowing and tilling, have been shown to alter the soil nutrient and moisture content (Monteiro & Lopes, 2007; Steenwerth & Belina, 2008; Sweet & Schreiner, 2010) and, since nutrient and water availability are tied to grapevine performance (vigour, yield, and vine growth), they ultimately influence the nitrogen contained in the fruit.

Previous reports have assessed the benefits rather than limitations of vineyard cover cropping (Ingels, Bugg, McGourty, & Christensen, 1998; Ingels, Scow, Whisson, & Drenovsky, 2005; Monteiro and Lopes, 2007; Steenwerth, Pierce, Carlisle, Spencer, & Smart, 2010; Wheeler et al., 2005). Cover cropping has long been popular as a weed control method for sustainable agricultural practises (anonymous; personal communications). Grape growers and winemakers wishing to practise soil conservation and sustainable agriculture, that lessen environmental costs to air and surface water quality, need up-to-date information on how these alterations to traditional field practises (i.e. mowing vs. tilling) might alter grape quality (Hobbs, Sayre, & Gupta, 2008; Papendick & Parr, 1997). In the long term, such vineyard floor approaches offer much in return for few production restrictions (Birkas, Jolankai, Kisic, & Stipesevic, 2008).

This study's primary objective was to compare YAN (mainly individual primary free amino acids and ammonia) of 'Cabernet Sauvignon' grapes grown on two rootstocks (high and low vigorous rootstocks; 110R and 420A), while observing the effect that three vineyard floor management regimes had on overall grape YAN. Secondly, YAN values from juiced (must) and chemically extracted grapes were compared, to correlate differences in measurements provided by analyses preparations, one a standard practise in the industry and the other more often used in research settings.

2. Materials and methods

2.1. Plant material

Random berry samples were collected from data vines, in 2005, at the UC Davis Oakville Experimental Vineyard in Napa county (Oakville, CA, USA; latitude 38° 25' 55" N, longitude 122° 24' 48"). The mean annual temperature at the vineyard during 2005 was 16.0 °C. The experimental plot was 46 rows, with 30 grapevines per row. Vines spacing was 1.8 m × 2.4 m, and row orientation was east–west. Rootstocks in this study were 110R (110 Richter; genetic origin of *V. berlandieri* × *V. rupestris*; high vigour) and 420A (420A Millardet et de Gasset; genetic origin of *V. berlandieri* × *V. riparia*; low vigour) grafted to scion 'Cabernet Sauvignon' clone 8. All study vines were planted in 1994. Due to the difference in vine canopy vigour imparted by the two rootstocks, the grapevines were trained to different position numbers. 110R rootstock vines were trained to twelve spur positions per cordon, while 420A rootstock vines were trained to eight spur positions per cordon. All vines were trained to a bilateral cordon with two

buds at each spur position. Additional details of this vineyard site were described by Steenwerth et al. (2010).

Three different vineyard floor management treatments were as follows: resident vegetation (RV), composed of forbs and annual grasses that were mowed and then tilled (control; current commercial vineyard floor management practise in CA, USA; abbreviated as RV + till), barley UC603 mowed in April (CC + mow), and barley UC603 mowed and then tilled in April (CC + till). Barley UC603 (*Hordeum vulgare*; a short stature barley) was planted over a two-year period (in November, 2003 and 2004). Plants identified in the resident vegetation treatment (RV + till) are listed by Steenwerth et al. (2010). All other vineyard practises (irrigation, fertilisation, pruning, etc.) were identical for all cover crop treatments (see Steenwerth et al., 2010 for details). Vines were drip-irrigated from June to August each year (ca. 105 l vine⁻¹ y⁻¹, in 2004 and 2005), and nitrogen fertiliser as potassium nitrate was added in June each year (ca. 6.7 kg N ha⁻¹ y⁻¹, 2004 and 2005). Experimental design was a randomized complete block design, with three blocks and two vines (replications) per block. All samples were harvested on September 20th, 2005, with a random composite sample value of 25 °Brix.

2.2. Reagents, chemicals and standards

All reagents, chemicals, and water used in this study were of analytical or high performance liquid chromatography (HPLC) grade, and were obtained from Sigma–Aldrich Co. (St Louis, MO, USA) unless indicated otherwise.

2.3. Sample preparation

Random berry samples were taken from data vines. Following common industry test preparation procedure, half of the berries were crushed and juiced to obtain juice samples, which were then kept frozen (–80 °C) prior to further analysis. Remaining berries were stored at –80 °C prior to processing. While they were still frozen, 50 were randomly selected, weighed, and fractionated into two fractions (skins and pulp: fraction a, versus seeds: fraction b) before being immediately placed in liquid nitrogen (Norco Inc., Nampa, ID, USA). Excess liquid nitrogen was evaporated off and the fraction weights were recorded. Liquid nitrogen frozen fractions were stored at –80 °C prior to extraction. Extraction was performed as described by Lee and Finn (2007) with minor changes, as described by Lee and Schreiner (2010). Briefly, an IKA M20 Universal mill (IKA works Inc., Wilmington, NC, USA) was used to liquid nitrogen–powder fractions A (FA). Fractions B (FB) were extracted as whole seeds. Whole berry extract values were obtained by summing values from FA and FB. All samples were extracted and re-extracted twice more (extracted a total of three times) in acidified methanol (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, USA) and re-dissolved in water (final volume of 25 ml). Aqueous extracts were used for each of the analyses. Samples were filtered through disposable 25 mm GD/X syringe filters (Whatman Inc., Florham Park, NJ, USA) before the ammonia, sugar, and organic acid analyses. Prior to free amino acid derivatization, samples were filtered with disposable Millex-FH syringe filters (Millipore, Bedford, MA, USA).

2.4. Ammonia determination

A SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA) and 1 cm disposable semi-micro cuvettes (Fisherbrand, Thermo Fisher Scientific Inc., Waltham, MA, USA) were used. The ammonia assay kit (AA0100) was obtained from Sigma–Aldrich Co. Manufacturer instructions were followed

Table 1
Summary of individual sugars, organic acids, ammonia, free amino acids, and YAN values of all 'Cabernet Sauvignon' juice and extract. 'Cabernet Sauvignon' clone 8 scion was grafted on the two rootstocks (110R and 420A; both $n = 9$). There is no significant evidence of interaction effect between rootstock and floor management ($p = 0.882$). There was evidence of significant difference between rootstock samples ($p = 0.022$). Values in parentheses are standard errors.

Sample form	Juice				Extracts (chemically extracted whole berries)			
	110R		420A		110R		420A	
Rootstock	110R		420A		110R		420A	
Total simple sugars (g/100 ml)	25.7 a	(0.20)	25.3 a	(0.24)	–	–	–	–
Glucose	12.9 a	(0.11)	12.7 a	(0.12)	–	–	–	–
Fructose	12.8 a	(0.10)	12.7 a	(0.12)	–	–	–	–
Total organic acids (g/100 ml)	0.57 a	(0.01)	0.72 b	(0.01)	–	–	–	–
Tartaric acid	0.42 a	(0.01)	0.59 b	(0.01)	–	–	–	–
Malic acid	0.15 a	(0.00)	0.13 a	(0.01)	–	–	–	–
Ammonia (mg N/l or kg)	47.8 aB	(2.71)	39.4 aB	(3.22)	21.7 aA	(1.54)	17.9 aA	(1.54)
Total free amino acids (mg N/l or kg) = FAN	146 aA	(6.27)	128 aA	(8.04)	236 bB	(8.60)	188 aB	(9.40)
1 Aspartic acid (ASP)	0.19 a	(0.07)	0.16 a	(0.01)	nd	–	nd	–
2 Glutamic acid (GLU)	2.37 aA	(0.11)	2.29 aA	(0.15)	2.25 aA	(0.21)	2.21 aA	(0.17)
3 Asparagine (ASN)	0.53 bA	(0.02)	0.46 aA	(0.01)	1.97 aB	(0.04)	1.86 aB	(0.05)
4 Serine (SER)	2.68 bA	(0.07)	2.19 aA	(0.06)	3.20 bB	(0.06)	2.79 aB	(0.09)
5 Glutamine (GLN)	2.91 bA	(0.16)	2.19 aA	(0.13)	5.10 bB	(0.11)	4.62 aB	(0.13)
6 Histidine (HIS)	2.46 bA	(0.14)	1.73 aA	(0.09)	3.22 aB	(0.18)	3.03 aB	(0.15)
7 Glycine (GLY)	0.36 bA	(0.02)	0.28 aA	(0.01)	0.56 aB	(0.05)	0.59 aB	(0.04)
8 Threonine (THR)	1.61 bA	(0.08)	1.27 aA	(0.05)	2.10 bB	(0.10)	1.76 aB	(0.08)
9 Citrulline (CIT)	0.38 aB	(0.03)	0.30 aB	(0.02)	0.08 aA	(0.00)	0.09 aA	(0.01)
10 Arginine (ARG)	15.0 bA	(0.71)	10.7 aA	(0.46)	16.8 bA	(0.86)	13.8 aB	(0.64)
11 Alanine (ALA)	4.88 bA	(0.29)	3.80 aA	(0.16)	4.55 aA	(0.10)	4.32 aB	(0.15)
12 Tyrosine (TYR)	0.31 bA	(0.01)	0.27 aA	(0.01)	0.67 aB	(0.03)	0.90 aA	(0.31)
13 Valine (VAL)	2.33 bA	(0.10)	1.91 aA	(0.06)	3.09 bB	(0.11)	2.63 aB	(0.09)
14 Methionine (MET)	0.24 aA	(0.02)	0.21 aA	(0.03)	0.27 aA	(0.02)	0.31 aA	(0.04)
15 Tryptophan (TRP)	0.79 aA	(0.14)	0.61 aA	(0.02)	2.45 aB	(0.06)	2.33 aB	(0.04)
16 Phenylalanine (PHE)	0.69 bA	(0.04)	0.57 aA	(0.03)	0.74 aA	(0.03)	0.69 aB	(0.02)
17 Isoleucine (ILE)	1.33 bA	(0.07)	1.07 aA	(0.04)	1.71 bB	(0.06)	1.42 aB	(0.07)
18 Leucine (LEU)	1.43 bA	(0.08)	1.09 aA	(0.04)	1.77 bB	(0.08)	1.41 aB	(0.06)
19 Lysine (LYS)	0.52 aA	(0.11)	0.31 aA	(0.01)	1.20 bB	(0.05)	1.04 aB	(0.03)
20 Hydroxyproline (HYP)	78.0 aB	(4.61)	73.8 aB	(7.70)	18.3 aA	(1.12)	16.2 aA	(0.98)
21 Proline (PRO)	26.8 aA	(1.37)	22.3 aA	(2.15)	166 bB	(7.99)	126 aB	(7.95)
YAN (mg N/l or kg)	88.8 bB	(3.44)	69.0 aA	(4.94)	73.5 bA	(2.44)	63.7 aA	(2.37)

Different lowercase letters indicate significant difference ($p \leq 0.05$) within the pair of rootstock samples within the extraction method (e.g. 110R juice vs. 420A juice). Different uppercase letters indicate significant difference ($p \leq 0.05$) between the two sample preparation methods (e.g. 110R juice vs. 110R extract). 'nd' stands for not detectable and '–' indicates value not measured. Simple sugars, organic acids, and free amino acids were determined by two HPLC methods. Ammonia was determined by enzymatic assay. YAN was calculated by summing ammonia and primary amino acid concentrations.

without alterations. Juice and chemically extracted samples were used for this determination. Analyses were conducted in duplicate.

2.5. HPLC analyses of sugars, organic acids, free amino acids and YAN calculation

Individual sugars and organic acids were analysed by an Agilent 1100 high performance liquid chromatography/diode array detector/refractive index detector (HPLC/DAD/RID; Agilent Technologies Inc., Palo Alto, CA, USA). Details of the simultaneous sugars and organic acids method were as described by Lee, Keller, Rennaker, and Martin (2009). A Rezex ROA-organic acid H+ (300 mm \times 7.8 mm, 8 μ m, Phenomenex, Torrance, CA, USA) analytical column was used for simultaneous analyses of organic acids and sugars. An 80% solution of dilute sulphuric acid (2.5 mM) to 20% acetonitrile was used as the mobile phase, under isocratic conditions, and the flow rate was 0.5 ml/min. The analytical run was 15 min. Column compartment was set to 55 °C. External standards of malic acid, tartaric acid, glucose, and fructose were used for identification and quantification. Malic acid standard was corrected for fumaric acid contamination. Juice samples were used for sugar and organic acid analyses.

Individual free amino acids were analysed by HPLC/DAD, set up with inline derivatization, using the autosampler (G1313A; Agilent Technologies Inc.) by *o*-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) prior to HPLC injection. All details of the HPLC column, mobile phase composition, and gradient conditions were as described by Schuster (1988) and Henderson, Ricker, Bidlingmeyer, and Woodward (2006), with modifications

described by Lee et al. (2009). Briefly, an Agilent 1100 series HPLC/DAD with Zorbax Eclipse AAA analytical (4.6 mm \times 150 mm, 5 μ m, Agilent Technologies Inc.) and a guard (4.6 mm \times 12.5 mm, 5 μ m, Agilent Technologies Inc.) column were used. OPA-derivatized amino acids were monitored at 338 nm and FMOC-derivatized amino acids were monitored at 262 nm. All free amino acids were identified and quantified by the external standard method. Internal standards were used for both derivatizations; norvaline was used for amino acid derivatized by OPA and sacrosine was used for amino acids derivatized by FMOC. Units for free amino acid were mg N/l or mg N/kg of whole berries (which will be referred to as mg N/kg for conciseness). Amino acid abbreviations used in this study are listed in Table 1. Juice and extract were both used for these analyses. Total free amino acids (FAN) were determined by summing the individual free amino acid values.

YAN values were determined by summing ammonia and individual primary free amino acids as recommended by Filipe-Ribeiro and Mendes-Faia (2007), Lee et al. (2009), and Lee and Schreiner (2010) that can be easily utilised by yeast (all individual free amino acids excluding HYP and PRO). YAN values were expressed as mg N/l (for juice) or kg (for extract).

2.6. Statistical analysis

Statistica for Windows version 7.1 (StatSoft Inc., Tulsa, OK, USA) was used for the statistical analyses. Differences among the different rootstock and treatment means were resolved using one-way analysis of variance (ANOVA) and *t*-test or Tukey Honest Significant Difference (HSD; $\alpha = 0.05$). The Pearson product

moment correlation coefficient (r) was used to assess the relationship between juice and chemically extracted samples.

3. Results and discussion

3.1. General

There were no significant interactions between rootstocks and floor management treatments ($p = 0.882$) for all measurements, so the interaction term was dropped. All measurements between the two rootstocks ($p = 0.022$) are compared and summarised in Table 1. There were no significant differences among the three floor managements ($p = 0.685$), but Tukey HSD did distinguish minor differences for each rootstock within the floor managements (Tables 2 and 3).

3.2. Simple sugars and organic acids by HPLC in juices

Rootstocks did have some effect on organic acids, but not on the simple sugars of the juices (Table 1). Glucose and fructose were found in all juices, with their ratio ranged from 0.99 to 1.01. Tartaric and malic acids were found in all juices, with tartaric being the predominant juice organic acid.

No significant difference was found, in glucose, fructose or total sugars, among the juice samples of either rootstock. Grapes from either rootstock had commercially acceptable sugar levels, both with total sugars above 25 g/100 ml, a measurement highly correlated with the % of soluble solids method commonly practised in wineries (Lee et al., 2009). Rootstock 420A juices were significantly higher in tartaric acid and total organic acids than were 110R juices, but showed no significant difference in malic acid.

3.3. Effects of sample preparation method on nitrogen-containing compounds: ammonia, individual free amino acids and FAN

Nitrogen-containing compounds in the juice and whole berry samples (which will be referred to as extracts to distinguish them from juices) showed more differences between rootstocks (Table 1) than they did among vineyard floor management treatments (Tables 2 and 3). Both rootstocks' juices were significantly higher in ammonia than were their corresponding extracts. Ammonia contents between the two rootstocks were similar between juice samples, and similar between the extract samples. The juice samples' higher (double) ammonia values might be due to the volatilisation of ammonia during stages of the extraction process (i.e. evaporation of the methanol under vacuum at 40 °C, or sonication). Ammonia values were significantly correlated between the two preparations (i.e. juice vs. extract) for each sample group ($r = 0.800$; $p \leq 0.05$), as demonstrated previously (Lee & Schreiner, 2010).

These 'Cabernet Sauvignon' samples had 21 free amino acids in their juices and 20 free amino acids in their extracts (Table 1). The identified free amino acids are listed here in elution order: ASP (detected in juices, not extracts), GLU, ASN, SER, GLN, HIS, GLY, THR, CIT, ARG, ALA, TYR, VAL, MET, TRP, PHE, ILE, LEU, LYS, HYP, and PRO.

In the juice samples, the top free amino acids (in decreasing order of %) were HYP (>53% of FAN), PRO (>17% of FAN), and ARG (>8% of FAN). In the extracts, this order changed and PRO (>67% of FAN) was the main free amino acid, followed by ARG \approx HYP (both >7% of FAN), indicating that reported individual free amino acid concentrations of grapes vary, depending upon the extraction method used. The apparent increase of PRO in the extracts, compared to the juices, could be from liquid nitrogen-powdering and acidified methanol extraction of the skin and pulp fraction (more

efficient extraction of the skin portion), which can be seen in Tables 2 and 3. ASP was not detected in extracts, and might be a result of the extraction process diluting the ASP concentration to a value below the limit of detection.

As grape HYP is predominately found in the pulp (as observed by Stines et al., 2000), the greater HYP% in juice samples may be due to the method by which these berries were prepared and the values expressed. Since juice samples were expressed only as the juiceable fraction of fruit (less dilute and with less mass considered in the calculation), HYP appeared in higher levels, leaving behind a large mass of solids (skins and seeds) which contrasted with whole berry extracts. Higher levels of PRO were found in both rootstock extracts compared to juices. The differences in extractable levels of PRO and HYP, between juices and extracts, did not impact YAN values, since those two amino acids are not primary free amino acids.

Stines et al. (2000) reported that 'Cabernet Sauvignon' grapes were dominant in PRO >> ARG > ALA \approx GLN in chemically extracted (mixture of methanol, chloroform and water) samples, though their values were expressed as μg of amino acids/g. Additionally, Stines et al. (2000) also separated their 'Cabernet Sauvignon' grapes into three fractions (skin, pulp, and seed) and observed that PRO was predominantly found in the skin fraction. After converting the data of Stines et al. (2000) into the units of this study (mg N/kg), the order of the major free amino acids expressed in their paper remains similar to that found in our 'Cabernet Sauvignon' berries (PRO > ARG > GLU \approx ALA).

3.4. Effects of rootstock on nitrogen-containing compounds: individual free amino acids, FAN, and YAN

Rootstock influenced concentrations of free amino acids in the juices and extracts. Juice samples (Table 1) from 110R rootstocks had significantly greater levels of ASN, SER, GLN, HIS, GLY, THR, ARG, ALA, TYR, VAL, PHE, ILE, and LEU. Rootstock 110R juices also had higher YAN values than had 420A juices, but FAN was not significantly different.

Extracts (Table 1) from 110R rootstocks were significantly higher in SER, GLN, THR, ARG, VAL, ILE, LEU, LYS, PRO, FAN and YAN than were 420A extracts. Although ASN, HIS, GLY, ALA, TYR and PHE were significantly different between the two rootstock juices, these individual free amino acids were not significantly different in the extracts. Conversely, LYS and PRO were significantly different between the two extracts, but not among the juices. Minor changes in free amino acid composition, due to pre-analysis sample preparation, have previously been demonstrated (Lee & Rennaker, 2011; Lee & Schreiner, 2010), and they re-emphasise that care should be exercised when comparing data in published literature.

The ability of a rootstock to influence free amino acid levels in grape juice had been demonstrated in 'Chardonnay' and 'Shiraz' (Holzapfel & Treeby, 2007; Huang & Ough, 1989; Treeby et al., 1998), but not in 'Cabernet Sauvignon' (Huang & Ough, 1989). Huang and Ough (1989) compared 'Cabernet Sauvignon' from Santa Ynez (CA, USA) grown on nine separate rootstocks, and found no significant differences in the free amino acid concentrations in the resulting juices. The mechanisms and means by which different rootstocks grafted to the same scion alter berry free amino acid levels are not well understood (Treeby et al., 1998). Future research is needed to resolve the relationships between nitrogen-containing compounds and the interactions of rootstocks and scions.

Rootstock 110R juices were significantly higher in CIT, HYP and YAN yet significantly lower in ASN, SER, GLN, HIS, GLY, THR, TYR, VAL, TRP, ILE, LEU, LYS, PRO and FAN when compared to their own extracts. These trends were slightly different in rootstock 420A juices and extracts. Rootstock 420A juice samples were significantly higher in CIT and HYP, and significantly lower in ASN,

Table 2

Results of the different cover crop treatments ($n = 3$) for rootstock 110R samples. There was no significant difference between the different cover crop treatments ($p = 0.866$), but individual variables were compared. Rootstock 110R samples from the different extraction methods are reported. Fraction A (FA) is the skin and pulp fraction and fraction B (FB) is the seed fraction. Values in parentheses are standard errors. Full names of the amino acid abbreviations are listed in Table 1.

Cover crop treatment Sample form	Control = RV + till				CC + mow				CC + till			
	Juice	Whole berry	FA	FB	Juice	Whole berry	FA	FB	Juice	Whole berry	FA	FB
Ammonia (mg N/l or kg)	48.2 (6.14)	21.3 (2.61)	20.6 (2.54)	0.69 (0.09)	42.8 (1.64)	19.1 (0.92)	18.4 (0.95)	0.67 (0.05)	52.5 (4.87)	24.8 (3.56)	24.0 (3.46)	0.81 (0.09)
Total free amino acids (mg N/l or kg) = FAN	140 (7.84)	219 (5.17)	211 (5.12)	7.91 (0.12)	139 (9.56)	232 (14.69)	225 (14.96)	6.83 (0.46)	158 (14.60)	257 (16.62)	249 (16.54)	7.82 (0.44)
ASP	0.12 (0.03)	nd	nd	nd	0.11 (0.03)	nd	nd	nd	0.34 (0.18)	nd	nd	nd
GLU	2.42 (0.11)	2.33 (0.12)	2.27 (0.12)	0.06 (0.01)	2.20 (0.18)	1.90 (0.37)	1.85 (0.36)	0.05 (0.01)	2.48 (0.28)	2.53 (0.51)	2.45 (0.52)	0.08 (0.01)
ASN	0.53 (0.04)	1.84 (0.04)	1.72 (0.04)	0.13 (0.00)	0.54 (0.04)	2.01 (0.04)	1.87 (0.03)	0.13 (0.00)	0.53 (0.04)	2.05 (0.05)	1.93 (0.05)	0.12 (0.01)
SER	2.66 (0.10)	3.27 (0.10)	3.16 (0.09)	0.11 (0.01)	2.66 (0.19)	3.15 (0.11)	3.04 (0.11)	0.11 (0.01)	2.74 (0.14)	3.18 (0.13)	3.06 (0.13)	0.12 (0.01)
GLN	3.11 (0.25)	5.46 (0.18)	5.26 (0.18)	0.20 (0.01)	2.79 (0.48)	4.83 (0.04)	4.61 (0.04)	0.22 (0.00)	2.84 (0.06)	5.02 (0.11)	4.80 (0.11)	0.21 (0.01)
HIS	2.47 (0.38)	3.24 (0.47)	2.92 (0.44)	0.32 (0.03)	2.27 (0.16)	3.04 (0.15)	2.76 (0.16)	0.28 (0.03)	2.63 (0.19)	3.39 (0.32)	3.01 (0.31)	0.39 (0.03)
GLY	0.37 (0.04)	0.56 (0.05)	0.54 (0.05)	0.02 (0.00)	0.36 (0.03)	0.51 (0.09)	0.47 (0.08)	0.04 (0.02)	0.36 (0.02)	0.61 (0.11)	0.58 (0.11)	0.03 (0.00)
THR	1.55 (0.12)	2.27 (0.24)	2.17 (0.23)	0.10 (0.01)	1.52 (0.19)	1.86 (0.06)	1.78 (0.07)	0.08 (0.01)	1.77 (0.10)	2.19 (0.12)	2.10 (0.11)	0.09 (0.02)
CIT	0.39 (0.06)	0.07 (0.01)	nd	0.07 (0.01)	0.38 (0.07)	0.07 (0.00)	nd	0.07 (0.00)	0.36 (0.04)	0.09 (0.00)	nd	0.09 (0.00)
ARG	14.0 (0.99)	16.8 (2.40)	16.6 (2.37)	0.22 (0.04)	14.0 (1.12)	15.8 (0.73)	15.6 (0.72)	0.18 (0.02)	17.0 (0.89)	17.8 (1.25)	17.5 (1.20)	0.25 (0.04)
ALA	4.86 (0.63)	4.77 (0.18)	4.54 (0.15)	0.23 (0.03)	4.75 (0.63)	4.33 (0.07)	4.12 (0.04)	0.21 (0.02)	5.03 (0.44)	4.56 (0.19)	4.30 (0.19)	0.27 (0.01)
TYR	0.30 (0.02)	0.62 (0.03)	0.57 (0.03)	0.05 (0.00)	0.30 (0.03)	0.69 (0.08)	0.64 (0.08)	0.04 (0.00)	0.33 (0.01)	0.71 (0.05)	0.65 (0.05)	0.05 (0.01)
VAL	2.25 (0.17)	3.08 (0.08)	2.95 (0.08)	0.14 (0.00)	2.36 (0.26)	3.18 (0.31)	3.03 (0.29)	0.15 (0.02)	2.37 (0.15)	3.00 (0.20)	2.85 (0.19)	0.15 (0.01)
MET	0.22 (0.04)	0.26 (0.02)	0.26 (0.02)	nd	0.24 (0.03)	0.27 (0.06)	0.27 (0.06)	nd	0.26 (0.02)	0.27 (0.03)	0.27 (0.03)	nd
TRP	1.07 (0.42)	2.30 (0.05)	2.07 (0.05)	0.24 (0.00)	0.62 (0.03)	2.55 (0.13)	2.29 (0.12)	0.26 (0.01)	0.67 (0.03)	2.50 (0.09)	2.23 (0.07)	0.27 (0.02)
PHE	0.73 (0.09)	0.80 (0.06)	0.75 (0.06)	0.05 (0.00)	0.66 (0.09)	0.70 (0.05)	0.65 (0.04)	0.05 (0.00)	0.69 (0.01)	0.72 (0.05)	0.68 (0.05)	0.04 (0.01)
ILE	1.27 (0.14)	1.73 (0.08)	1.66 (0.08)	0.07 (0.00)	1.37 (0.16)	1.75 (0.12)	1.68 (0.12)	0.07 (0.00)	1.35 (0.09)	1.65 (0.12)	1.58 (0.12)	0.07 (0.01)
LEU	1.33 (0.14)	1.74 (0.10)	1.65 (0.09)	0.09 (0.01)	1.47 (0.18)	1.79 (0.15)	1.69 (0.14)	0.10 (0.01)	1.48 (0.13)	1.79 (0.20)	1.68 (0.19)	0.11 (0.01)
LYS	0.73 (0.32)	1.15 (0.05)	1.15 (0.05)	nd	0.40 (0.04)	1.25 (0.13)	1.25 (0.13)	nd	0.43 (0.03)	1.21 (0.09)	1.21 (0.09)	nd
HYP	75.9 (6.30)	20.2 (1.90)	18.1 (2.09)	2.07 (0.33)	73.7 (6.72)	15.5 (0.70)	13.9 (0.68)	1.53 (0.25)	84.3 (11.77)	19.4 (2.15)	17.5 (2.07)	1.87 (0.20)
PRO	24.0 (1.86)	146 (6.34)	142 (6.45)	3.75 (0.12)	26.8 (2.60)	167 (13.92)	164 (13.94)	3.26 (0.30)	29.7 (2.07)	184 (13.21)	180 (13.23)	3.61 (0.18)
YAN (mg N/l or kg)	88.5 (6.66)	73.6 (4.90)	70.8 (4.65)	2.79 (0.24)	81.8 (3.93)	68.8 (1.18)	66.1 (1.23)	2.72 (0.14)	96.1 (5.55)	78.0 (4.97)	74.9 (4.81)	3.15 (0.17)

No letters after averages indicate no significant difference. 'nd' stands for not detectable and '-' stands for not calculated.

Table 3

Results of the different cover crop treatments ($n = 3$) for rootstock 420A. There was no significant difference between the different cover crop treatments ($p = 0.896$), but individual variables were compared. Samples from Rootstock 420A. Fraction A (FA) is the skin and pulp fraction and fraction B (FB) is the seed fraction. Values in parentheses are standard errors. Full names of the amino acid abbreviations are listed in Table 1.

Cover crop treatment	Control = RV + till								CC + mow								CC + till							
	Juice		Whole berry		FA		FB		Juice		Whole berry		FA		FB		Juice		Whole berry		FA		FB	
Ammonia (mg N/l or kg)	43.4	(4.73)	20.1	(2.39)	19.5	(2.31)	0.63	(0.11)	36.3	(9.28)	16.6	(3.61)	16.1	(3.47)	0.49	(0.14)	38.5	(1.55)	17.1	(2.47)	16.6	(2.35)	0.57	(0.12)
Total free amino acids (mg N/l or kg) = FAN	137	(14.32)	188	(8.40)	182	(7.68)	5.88	(0.75)	123	(22.07)	185	(30.94)	179	(29.95)	6.12	(0.99)	123	(4.55)	191	(4.68)	184	(4.75)	6.84	(0.24)
ASP	0.15	(0.01)	nd	–	nd	–	nd	–	0.16	(0.01)	nd	–	nd	–	nd	–	0.18	(0.02)	nd	–	nd	–	nd	–
GLU	2.39	(0.27)	2.24	(0.47)	2.16	(0.45)	0.07	(0.02)	2.16	(0.22)	2.17	(0.30)	2.10	(0.30)	0.07	(0.02)	2.33	(0.37)	2.23	(0.16)	2.12	(0.17)	0.10	(0.01)
ASN	0.48	(0.00)	1.94	(0.07)	1.81	(0.07)	0.13	(0.00)	0.47	(0.02)	1.72	(0.04)	1.57	(0.01)	0.15	(0.03)	0.45	(0.02)	1.92	(0.06)	1.78	(0.06)	0.14	(0.01)
SER	2.19	(0.06)	2.76	(0.15)	2.66	(0.14)	0.10	(0.01)	2.21	(0.18)	2.65	(0.13)	2.56	(0.13)	0.09	(0.01)	2.16	(0.07)	2.97	(0.16)	2.84	(0.15)	0.13	(0.00)
GLN	2.33	(0.23)	4.53	(0.11)	4.31	(0.11)	0.21	(0.00)	2.21	(0.34)	4.70	(0.33)	4.48	(0.34)	0.22	(0.02)	2.05	(0.15)	4.63	(0.27)	4.40	(0.26)	0.22	(0.01)
HIS	1.71	(0.09)	2.89	(0.26)	2.59	(0.26)	0.31	(0.02)	1.67	(0.28)	2.81	(0.03)	2.50	(0.02)	0.31	(0.04)	1.79	(0.09)	3.39	(0.32)	2.96	(0.28)	0.43	(0.06)
GLY	0.27	(0.00)	0.54	(0.07)	0.52	(0.07)	0.03	(0.00)	0.30	(0.02)	0.59	(0.07)	0.56	(0.07)	0.03	(0.01)	0.28	(0.01)	0.63	(0.08)	0.60	(0.08)	0.03	(0.01)
THR	1.27	(0.09)	1.75	(0.11)	1.67	(0.10)	0.08	(0.01)	1.28	(0.16)	1.59	(0.20)	1.52	(0.20)	0.07	(0.01)	1.25	(0.05)	1.94	(0.05)	1.84	(0.05)	0.09	(0.01)
CIT	0.30	(0.04)	0.08	(0.00)	nd	–	0.08	(0.00)	0.33	(0.02)	0.08	(0.01)	nd	–	0.08	(0.01)	0.28	(0.05)	0.12	(0.02)	nd	–	0.12	(0.02)
ARG	11.1	(0.57)	13.8	(0.73)	13.6	(0.71)	0.14	(0.03)	10.6	(1.43)	12.4	(1.11)	12.3	(1.07)	0.13	(0.04)	10.4	(0.25)	15.2	(1.09)	15.1	(1.07)	0.14	(0.02)
ALA	3.87	(0.06)	4.25	(0.09)	4.00	(0.09)	0.25	(0.01)	3.73	(0.55)	4.12	(0.40)	3.88	(0.41)	0.24	(0.03)	3.81	(0.08)	4.58	(0.15)	4.28	(0.14)	0.30	(0.02)
TYR	0.25	(0.01)	0.56	(0.03)	0.51	(0.03)	0.04	(0.01)	0.28	(0.03)	1.50	(0.95)	1.46	(0.95)	0.04	(0.00)	0.29	(0.03)	0.64	(0.06)	0.61	(0.06)	0.04	(0.00)
VAL	1.86	(0.06)	2.53	(0.11)	2.40	(0.10)	0.13	(0.01)	1.92	(0.10)	2.52	(0.12)	2.41	(0.12)	0.11	(0.01)	1.96	(0.18)	2.83	(0.19)	2.68	(0.18)	0.15	(0.01)
MET	0.22	(0.05)	0.30	(0.05)	0.30	(0.05)	nd	–	0.26	(0.08)	0.32	(0.08)	0.32	(0.08)	nd	–	0.15	(0.01)	0.31	(0.09)	0.31	(0.09)	nd	–
TRP	0.63	(0.01)	2.27	(0.09)	2.02	(0.10)	0.25	(0.00)	0.60	(0.01)	2.31	(0.09)	2.04	(0.07)	0.27	(0.02)	0.59	(0.04)	2.40	(0.06)	2.14	(0.06)	0.26	(0.01)
PHE	0.58	(0.03)	0.67	(0.03)	0.63	(0.03)	0.04	(0.00)	0.56	(0.08)	0.68	(0.04)	0.64	(0.04)	0.04	(0.01)	0.56	(0.03)	0.72	(0.02)	0.68	(0.02)	0.04	(0.00)
ILE	1.02	(0.03)	1.34	(0.05)	1.28	(0.06)	0.06	(0.00)	1.08	(0.04)	1.33	(0.08)	1.27	(0.07)	0.06	(0.01)	1.12	(0.13)	1.58	(0.16)	1.51	(0.15)	0.07	(0.01)
LEU	1.03	(0.03)	1.32	(0.08)	1.24	(0.07)	0.09	(0.01)	1.11	(0.05)	1.37	(0.03)	1.29	(0.03)	0.07	(0.00)	1.13	(0.12)	1.55	(0.14)	1.46	(0.13)	0.10	(0.01)
LYS	0.30	(0.01)	0.98	(0.02)	0.98	(0.02)	nd	–	0.31	(0.01)	1.02	(0.02)	1.02	(0.02)	nd	–	0.33	(0.02)	1.13	(0.07)	1.13	(0.07)	nd	–
HYP	83.8	(16.69)	17.8	(2.24)	16.1	(2.02)	1.69	(0.23)	71.3	(17.51)	15.0	(1.94)	13.1	(1.57)	1.94	(0.38)	66.3	(6.71)	15.7	(0.74)	13.9	(0.67)	1.81	(0.09)
PRO	20.9	(3.90)	126	(4.71)	123	(4.25)	2.17	(0.52)	20.1	(1.62)	126	(26.84)	124	(26.21)	2.18	(0.67)	25.8	(5.34)	127	(3.90)	124	(4.03)	2.67	(0.19)
YAN (mg N/l or kg)	75.3	(5.66)	64.8	(3.83)	62.2	(3.67)	2.65	(0.17)	62.3	(14.70)	60.5	(5.58)	58.0	(5.47)	2.49	(0.25)	69.5	(1.45)	65.9	(3.65)	63.0	(3.53)	2.94	(0.15)

No letters after average indicate no significant difference. 'nd' stands for not detectable and '–' stands for not calculated.

SER, GLN, HIS, GLY, THR, ARG, ALA, TYR, VAL, TRP, PHE, ILE, LEU, LYS, PRO and FAN than were extracts of 420A. Extraction was more effective at removing FAN than was juicing from the fruit. Overall, FAN values were highly correlated between a rootstock's juice and extract ($r = 0.853$; $p \leq 0.05$).

Rootstock also influenced the YAN content of juices and extracts (Table 1). 110R juices and extracts were both higher in YAN than were 420A juices and extracts. No difference in YAN was observed between juices and extracts of 420A, showing the extraction method's influence upon YAN values. Only the 110R samples' greater YAN contents allowed a significant difference in extraction to be observed.

The YAN values, between juice and extract, were highly correlated ($r = 0.843$; $p \leq 0.05$) and the linear relationship between the two preparations has been pointed out by Lee and Schreiner (2010). As mentioned above, ammonia contributing to YAN was higher (54% for 110R juice; 57% for 420A juice) in juice samples than in chemically extracted samples (30% for 110R extract; 28% for 420A extract).

Both rootstock juices and extracts (Table 1) were well below the YAN value commonly recommended by the industry and generally regarded as a baseline value by researchers, i.e. 140 mg/l (Bely et al., 1990; fermentation temperature held at 24 °C using *Saccharomyces cerevisiae* K1 ICV-INRA). Again, both rootstocks' juices and extracts were considered nitrogen-deficient if one intended to use *S. cerevisiae* PYCC 4072, since this yeast requires a minimum of 267 mg N/l to ferment to dryness (Mendes-Ferreira, Mendes-Faia, & Leao, 2004). All samples contained YAN below 90 mg N/l or kg, which indicates that these juices would need supplementation before the start of alcoholic fermentation. It is also possible that the vines need nitrogen fertilisation as juice nitrogen status and ammonia have been highly correlated with vine nitrogen status (Van Leeuwen et al., 2000).

3.5. Comparison of values among the different vineyard floor treatments

Again, no significant difference was found when the three different vineyard floor treatments were compared ($p = 0.866$ for 110R samples and $p = 0.896$ for 420A samples). The treatments are summarised and separated by rootstock (Table 2–110R, Table 3–420A). Only three free amino acids (ASN, GLN, and CIT) showed significant differences among the three vineyard floor management treatments in 110R extracts, trends that were not revealed in 110R juices. There was a significant difference in SER and LEU from 420A fraction B extracts among the three vineyard floor treatments. Although soil water content and soil respiration were altered by floor management treatments (Steenwerth et al., 2010), these initial shifts in soil processes had little influence on grape YAN. In particular, soil water content in late spring and summer, under the mown cover crops (i.e., no-till), was 2% ($\text{g H}_2\text{O g}^{-1}$ soil $\times 100$) lower than that of the tilled treatments, due to re-growth and tillering by the barley cover crop, and soil respiration rates increased immediately after tilling or mowing had occurred (Steenwerth et al., 2010). All individual free amino acids were present at higher concentrations in FA than in FB, except CIT (both rootstocks and all vineyard floor treatments). CIT was present at a non-detectable level in FA, possibly due to dilutions introduced by the extraction procedure. Primary free amino acid contributed 68.3–74.0% to YAN, as reported by others (Filipe-Ribeiro & Mendes-Faia, 2007; Lee & Schreiner, 2010; Lee et al., 2009). The majority of the YAN was contributed from FA (Tables 2 and 3), accounting for 95% of YAN. These results indicate that no-till management did not impact grape nitrogen status in these fields.

Stines et al. (2000) reported that 'Cabernet Sauvignon' seeds contributed 8.5% to the total grape berry FAN content, which was

higher than the levels observed in this study (3.8–4.5% of whole berries FAN). This difference is partially due to the extraction method used. Stines et al. (2000) liquid nitrogen-powdered their seed fractions and used a solvent mixture (mentioned in Section 3.4) different from that in our whole seed and acidified methanol extraction. The analytical method used will also contribute to the differences in determining individual free amino acids, since Stines et al. (2000) used a derivatization and HPLC condition different from that used for this study.

Our results also differed from what Wheeler et al. (2005) observed in their cover crop treatments. Wheeler et al. (2005) observed reduced ammonia in their 'Cabernet Sauvignon' grapes from chicory cover crops that were mowed (permanent) or herbicide-treated, compared to three other treatments (bare soil – herbicide, bare soil – cultivated, and sawdust). Although, the ammonia values were significantly lower in the two chicory treatments (both mowed and herbicide-treated), in a sensory evaluation of the resulting wines, those from the chicory treatments were found more favourable than were wines from the other three treatments (Wheeler et al., 2005).

Based on this current study, grape nitrogen compounds are chiefly influenced by rootstock/scion combination, rather than by vineyard floor manipulation. However, the vineyard floor treatment were only existent for two years prior to sampling and, given that grape is a woody, perennial plant with vine nutrient reserves, one might anticipate longer-term cumulative effects of vineyard floor management. Again, these experimental differences highlight the results being affected by the sample preparation techniques used (Lee & Schreiner, 2010). In the case of 'Cabernet Sauvignon' clone 8, a more vigorous rootstock could be a tool for obtaining a higher grape YAN. Additional work, linking certain grape nitrogen compounds to specific wine volatile formations (Hernandez-Orte, Cacho, & Ferreira, 2002; Miller, Wolff, Bisson, & Ebeler, 2007; Ugliano et al., 2009; Vilanova et al., 2007), might allow the wine-makers to manipulate grape nitrogen concentrations by rootstock selection, which could enhance future winemaking strategies.

4. Conclusion

'Cabernet Sauvignon' clone 8 grown on two different rootstocks (110R and 420A) exhibited altered grape nitrogen compounds (more than by vineyard floor management practises), suggesting that vineyard managers can utilise barley and mowing (no-tillage) without impacting grape nitrogen compounds, sugars or organic acids, at least in the early period after adoption. The more vigorous rootstock here, 110R, had higher levels of YAN and certain free amino acids than had 420A, although grapes from both rootstocks would likely require nutrient supplementation prior to alcoholic fermentation. There were also significant differences among the nitrogen-containing compounds, depending on extraction method, which emphasises the importance of unifying extraction methods before comparing values. This cover crop research is only in the initial phase at this site, and it will be interesting to discover if, in future years, the no-tilling (mowing) vineyard floor managements eventually alter grape nitrogen profiles.

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