Vitis **52** (2), 97–104 (2013)

Vineyard and winery indicators of 'Shiraz' must fermentation behaviour

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Summary

Nitrogen supply and rootstock have important consequences for the composition and quantity of nitrogenous compounds in the must, both of which impact on fermentation rate and wine quality. In the Sunraysia district (SE Australia), musts prepared from 'Shiraz' grapes from vines grafted onto three rootstocks and supplied with five different nitrogen (N) regimes were fermented to dryness. Leaf N at flowering and veraison, and berry and juice total N at harvest was influenced by N supply, but the juice total assimilable amino N pool was less sensitive. Consumption rate of soluble solids during fermentation was strongly and positively linearly related to %N in the petioles at veraison. The relationship described could be the basis of a tool to provide oenologists with timely data before harvest and receival on likely fermentation behaviour of specific parcels of grapes, and provide viticulturalists with another recognisable developmental stage to assess the efficacy of vineyard N management strategies within a season.

K e y w o r d s : Nitrogen, must, amino acids, fermentation, wine.

Introduction

In a previous paper we described the response of 'Shiraz' vines grafted on three different rootstocks to variations in N fertiliser supply timing and amount supplied (Holzapfel and Treeby 2007). We were particularly interested in the impact of N supply and rootstock on the concentrations of free primary amino acids in berries because this is the principle source of reduced nitrogen that yeast use during anaerobic fermentation (Ingledew *et al.* 1987). Must free primary amino N and ammonium are collectively referred to as yeast assimilable N or YAN. Yeast typically need about 130 mg free primary amino N/L of white must to complete a fermentation through to dryness (*i.e.* residual sugar less than the organoleptic threshold) (Agenbach 1977), the requirements for red musts are unknown. Low must N is associated with sluggish and stuck ferments (Arias-Gil *et al.* 2007), and high N musts ferment too quickly (Monteiro and Bisson 1991), requiring extra management (e.g. cooling). Between the extremes the relationship between must free primary amino N and the rate of sugar consumption by yeast is quantitative (Monk *et al.* 1987). Red grape musts are fermented on skins to a predetermined level of sugar depletion (generally 50 %); the rate of fermentation then has implications for the length of time that extraction of minerals and secondary metabolites from skins can occur. Berry and must free primary amino N levels have other direct effects on final wine composition: H_2S production is more likely if free primary amino N levels are limiting (Gardner *et al.* 2002), and urea and hence ethyl carbamate production is more likely if free primary amino N levels are too high (Ough *et al.* 1989). In addition, the carbon skeletons of de-aminated amino acids are involved in the formation of higher alcohols (Äyräpää 1971) and hence aroma. Below a particular level higher alcohols contribute positively to aroma, but above that higher alcohols detract from the aroma profile (Vilanova *et al.* 2007). Thus, must free primary amino N levels at harvest are important for the logistics of vinification and for the composition of the final wine.

Deficiencies of YAN in musts are readily corrected by addition of di-ammonium phosphate (DAP) (Mendes-Ferreira *et al.* 2009). Australian grape musts are generally considered to be relatively low with respect to YAN (Weeks and Henschke 1999 and references therein), and so the use of DAP is common in Australian wineries. However, additions are frequently made without reference to the level of endogenous YAN present, running the risks referred to above. Indiscriminant use of DAP may be due to the capital intensive nature of the instrumentation needed to estimate major YAN components, such as arginine, and the associated skill level and time required. Dukes and Butzke (1998) described a spectrophotometric method based on *ortho*-phthaldialdehyde, but the issue of timeliness remains. A timely method of predicting fermentation behaviour, as affected by YAN, would be useful to oenologists to match DAP additions to the level of YAN deficiency.

Previously we showed that concentrations of free primary amino N in 'Shiraz' berries were principally a function of N supply during the flowering to veraison period, but that free primary amino N levels in grapes grown on vines grafted on 'Schwarzmann' (*Vitis riparia* × *V. rupestris*) were also influenced by N supply the previous autumn (Holzapfel and Treeby 2007). We also presented data across seasons which suggested that assimilable amino N in the berries at harvest was linearly related to % N in tissue of the leaf (blade or petiole) opposite the basal bunch at veraison. In this paper we describe the effects that rootstock choice and management of N in the vineyard has on fermentation behaviour of a single season's crop. We use

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those data to evaluate the relationship between that behaviour and various indices of vine and grape berry N status to identify a suitable predictor of fermentation behaviour and a suitable benchmark value for that predictor, as well as the implications of various N supply management strategies. The overall aim was to provide oenologists with timely predictive tools to manage fermentation as influenced by YAN, and to provide viticulturalists a quantitative target by which to assess vineyard N supply management programs not just in relation to yield, but also in relation to berry composition.

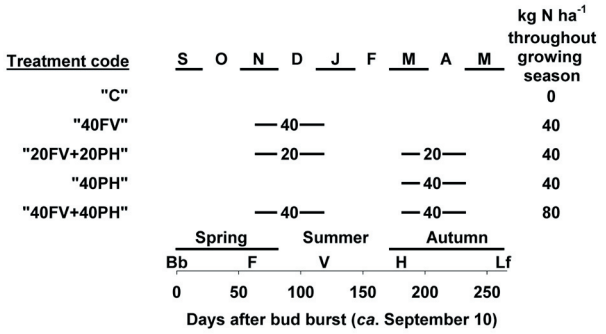

Fig. 1: Nitrogen supply treatments imposed on grafted drip-irrigated 'Shiraz' vines from spring 1993 to autumn 1996. The horizontal bars for each treatment indicate the start and finish of the N supply period for that treatment, and the number of kg of N·ha^{-1} supplied during each supply period is indicated in the middle of each bar. The start and end of each month and climatic season are indicated by the discontinuous lines above and below the N supply periods, respectively, and the approximate dates of the major phenological milestones are also indicated: **Bb**, budburst; **F**, flowering; **V**, veraison; **H**, harvest; **Lf**, leaf fall. The treatment codes used in the text are indicated on the left.

Material and Methods

Field trial set-up: The set up and conduct of the trial have been described in detail previously (Holzapfel and Treeby 2007). Briefly, the Shiraz vines used were planted in 1991 at a spacing of 1.5 m within rows and 3 m between rows. The vines were grafted on the rootstocks 'Teleki 5C' (*V. berlandieri* × *V. riparia*), 'Schwarzmann' (*V. riparia* × *V. rupestris*) and 'Ramsey' (*V. champini*), trained on 2 bilateral cordons, and spur pruned in winter. The vines were drip irrigated, with two 3 L·hour^{-1} inline dripper outlets per vine, 75 cm apart. The soil was a Moorook sandy loam (calcareous earth: Gc 1.12) (Northcote 1989, Stace *et al.* 1968), dominated by coarse sand, with the gravel fraction increasing with depth. The vineyard was located at Gol Gol, south west NSW, Australia (34°S, 142°E), in the grape growing region of Sunraysia, a warm irrigated region with low rainfall (mean January temperature and annual rainfall of 24.2 °C and 298 mm, respectively).

'Shiraz' vines on each of the rootstocks were planted in discrete areas (5 rows of 'Ramsey' next to 5 rows of 'Schwarzmann', which were next to 4 rows of 'Teleki 5C'). Each trial plot consisted of 5 vines within a row, with sampling and measurements only being carried out on the 3 middle vines. Within each of the 'Ramsey' and 'Schwarzmann' areas a 5 × 5 Latin square was set up, and a 4 × 5 Youden square was set up in the 4 rows of the 'Teleki 5C' vines. The drip irrigation system had been modified to impose 5 N supply treatments (Fig. 1) which allowed comparisons to be made between rate (0, 40 or 80 kg N·ha^{-1}/season) and timing (flowering to veraison, versus post-harvest to leaf fall, or both). The N was applied as ammonium nitrate through the drip system on a weekly basis in 8 equal amounts in each of the supply periods. The fertiliser was injected in the last 2 h of an irrigation cycle, which, depending on the time of the season, generally lasted 4 to 8 h. The treatments were applied for 3 growing seasons beginning in November, 1993, and finishing in May, 1996. The grapes used for the study reported in this paper were from the final season, viz 1995/96. By flowering in that season (November 1995), the C, 40 FV, 20 FV + 20 PH, 40 PH and 40 FV + 40 PH treatments had received a total of 0, 80, 80, 80 and 160 kg N·ha^{-1}, respectively, and by veraison (January 1996) 0, 120, 100, 80 and 200 kg N·ha^{-1}, respectively, since the start of the experiment in November 1993.

Leaf tissue sampling and preparation for analysis: Leaves (petioles and laminae) from opposite basal bunches were sampled from the middle 3 vines in each plot at flowering (50 % capfall) and veraison (50 % of berries soft). The leaves were washed in lightly acidified water with a few drops of phosphate-free detergent per litre, rinsed in 2 changes of de-ionised water, blotted dry, separated into petioles and blades and dried in paper bags in a fan forced oven at 40 °C. The dried material was ground in a hammer mill to a fine powder.

Small-scale winemaking: The grapes from each plot were harvested at similar maturity levels on 15 March 1996. The grapes were transported to CSIRO Plant Industry's small scale winery at Merbein in north west Victoria, and stored at 4 °C until they were processed two days later. To reduce the field variation the grapes from each treatment were bulked and 5 replicates were created for the winemaking process. Thus, 75 separate ferments were conducted. The winemaking procedure was similar to that described by Kerridge (1983) with the exception that DAP was not added to any must. About 15 kg of must was placed into a 20 L fermenter, yeast (Lalvin ICV D254) was added, and the must was fermented at a temperature of 24 °C. Consumption of total refractible soluble solids (TSS) was used as an indicator of fermentation rate. The skins were pushed down twice per day until pressing off, which was carried out at about 50 % TSS depletion (6 to 13 d later). The fermenting juice was then syphoned into 9.5 L glass bottles for further fermentation to dryness (≤ 0.2 % residual sugar, although this was not reached by all ferments), determined with the Clinitest. After about 5 weeks the first racking off was carried out, and the wine was stored at 15 °C for 10 d until the second racking off. The wine was then stored at 4 °C (for further 7 weeks) for cold stabilisation and tartrate removal (total time 14.5 weeks).

At this point samples of the finished wine were taken for residual N and residual sugar determination.

Grape berry sampling and preparation for analysis: Three d before harvest 10 bunches were taken from the middle three vines of each replicate plot. Five berries from each bunch were taken to form a composite sample of 50 berries for each replicate plot. The berries were frozen and stored at -70 °C until they were macerated with a homogeniser (Ultra-Turrax® T25 Basic, IKA Werke GmbH, Staufen, Germany). Approximately 1 g of grape puree was taken and placed into a 10 mL centrifuge tube with 10 mL of 50 % ethanol, and was used for colour determination.

After crushing and destemming each winemaking replicate, approximately 10 mL of must was spun at 3,000 g for 10 min and supernatant retained for titratable acidity (TA), pH and total refractable soluble solids (TSS) determination. Must samples were also frozen and stored at -70 °C for later analysis of total N and free amino acid concentrations.

Grape juice and wine soluble solids, pH and TA: TSS were determined with a temperature-compensated digital refractometer (Erma Inc., Tokyo, Japan), and pH and TA were measured with an automatic titrator (Radiometer Analytical A/S, Copenhagen, Denmark). TSS consumption was estimated by correcting apparent Brix for alcohol interference according to an equation published by Son *et al.* (2009).

Grape phenolics and colour: Determinations of berry total anthocyanins and phenolics were conducted according to Somers and Evans (1974) at the Australian Wine Research Institute (AWRI), Adelaide, South Australia.

Petiole, berry, juice, must and wine total N and must free amino acids: Nitrogen in dried ground leaf tissue sampled at flowering and veraison, whole berries and juice sampled at harvest and the final wine were determined with a LECO FP2000 N analyser (furnace temperature 1,200 °C) (LECO Corporation, St Joseph, MI, USA).

To measure free amino acids, the frozen juice samples were thawed and filtered (0.45 µm) and the filtrate diluted 1:20 with 0.25M borate buffer. The concentrations of free amino acids in the diluted filtrate were determined by derivatising with 9-fluroenylmethyl chloroformate (Hynes *et al.* 1991), separating on a reverse phase column (150 x 4.6 mm ID, ODS Hypersil, Keystone Scientific Inc., Bellefonte, PA, USA) using a HPLC (LC1150, GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia) and quantifying against standards with a fluorescence detector (GBC LC1250). Twenty one free amino acids were quantified, but levels of 3 of these were frequently below or only marginally higher than the detection limit, and these data were not included in any of the calculations described below. Levels of total free amino N, assimilable amino N and non-assimilable amino N were calculated from the concentrations of each of the remaining 18 free amino acids and the number of N atoms, the number of α amino N atoms and the number of non-α amino N atoms of each amino acid. Ammonium is also a source of N for yeast (Cruz *et al.* 2002), but the HPLC protocol used could not detect ammonium, and so the assimilable amino N pool referred to in this paper is an underestimate of YAN.

Residual sugar in the final wines: Measurement of residual sugar (RS) in the final wine was carried out with an enzymatic kit for D-glucose/D-fructose (UV-method, Boehringer Mannheim), and was conducted at the AWRI.

Data analysis: Statistical analyses were conducted using GenStat Release 7.2 (Rothamsted Experimental Station). The impact of N supply on berry composition were analysed as follows: data for 'Shiraz' on 'Ramsey' or 'Schwarzmann' were analysed as 5 × 5 Latin Squares; data for 'Shiraz' on 'Teleki 5C' were analysed as a 5 × 4 Youden Square. Significant ($p \leq 0.05$) responses for Shiraz on each rootstock were identified in an analysis of variance, and significant differences between means identified using least significant differences, and between groups of means using orthogonal contrasts. Fermentation behaviour and final wine composition were analysed using the winemaking replicates (ANOVA without blocking). Where needed, data were transformed according to the λ value generated by Genstat's "Best λ" procedure to satisfy the assumptions underpinning analysis of variance. Orthogonal contrasts were used to partition overall treatment effects into effects due to N timing and effects due to N rate. Means presented in tables and figures were calculated from the untransformed data, but mean separations were carried out on means calculated from the transformed data if a transformation had been necessary. Effects referred to as 'significant' had an F ratio with a probability of ≤ 0.05 in an analysis of variance.

The correlations between fermentation behaviour and the vine, berry and juice N measurements were calculated using the field and winemaking means.

Results

Vine N status at flowering and veraison: Indicators of vine N status reflected the timing and to a lesser extent, the amount of N supplied, but the magnitude of the effects appeared to differ across the rootstocks (Tab. 1). Vines on 'Teleki' were generally less responsive to N supply compared to vines on 'Schwarzmann' and 'Ramsey'. Across all rootstocks, vines that received N during the postharvest period tended to have higher % N at flowering, and vines that received N during the flowering to veraison period had higher % N at veraison compared to vines that did not receive N during that period. Vines on 'Schwarzmann' were the exception to the latter generalisation: petiole % N at veraison for vines on this rootstock indicated that some benefit was still being derived from the postharvest N supply in Season 1994-95 at veraison in Season 1995-96.

Berry composition: Nitrogen supply affected the fresh weight of berries produced on vines growing on 'Schwarzmann', but the extent of the effect was not large,

Table 1

Effect of N supply on the N status leaf petiole and blade of leaves opposite the basal bunches at flowering and veraison for 'Shiraz' on 3 different rootstocks. Within columns and rootstocks means followed by the same superscript are not significantly different at P = 0.05. Values presented are means (n = 4 for 'Teleki' and n = 5 for 'Schwarzmann' and 'Ramsey')

Rootstock	Treatment	Petioles		Laminae	
		Flowering	Veraison	Flowering	Veraison
		DM %N			
Teleki 5C	C	0.85[a]	0.48[a]	2.45[a]	1.78[a]
	40FV	0.91[ab]	0.50[b]	2.57[ab]	1.97[b]
	20FV+20PH	0.96[b]	0.49[ab]	2.63[ab]	1.88[ab]
	40PH	0.95[ab]	0.48[a]	2.67[b]	1.81[a]
	40FV+40PH	0.95[ab]	0.51[b]	2.66[b]	2.01[b]
Schwarzmann	C	0.77[ab]	0.46[a]	2.58[bc]	1.70[a]
	40FV	0.73[a]	0.52[bc]	2.43[a]	2.02[b]
	20FV+20PH	0.74[a]	0.49[ab]	2.51[ab]	1.81[a]
	40PH	0.80[b]	0.54[c]	2.64[c]	1.85[a]
	40FV+40PH	0.78[b]	0.63[d]	2.63[c]	2.08[b]
Ramsey	C	0.78[a]	0.52[a]	2.87[ab]	1.94[a]
	40FV	0.97[b]	0.62[cd]	2.80[a]	2.26[c]
	20FV+20PH	0.95[b]	0.59[bc]	2.94[b]	2.19[bc]
	40PH	0.98[b]	0.55[ab]	2.98[b]	1.99[ab]
	40FV+40PH	0.92[ab]	0.65[d]	2.96[b]	2.44[c]

Table 2

Effect of N supply on berry weight and juice composition of 'Shiraz' on 3 different rootstocks. Within columns and rootstocks means followed by the same superscript are not significantly different at P = 0.05. Values presented are means (n = 4 for 'Teleki' and n = 5 for 'Schwarzmann' and 'Ramsey')

Rootstock	Treatment	Berry weight	TSS	Titratable acidity	pH	K^+	Total phenolics	Anthocyanin
		g·berry^{-1}	g·100 g^{-1}	g·L^{-1}		g·L^{-1}	mg·g^{-1} FW	
Teleki 5C	C	1.33	23.5	4.58	3.59[a]	0.82	1.69	0.86[a]
	40FV	1.30	23.0	5.00	3.45[b]	0.84	1.66	0.92[a]
	20FV+20PH	1.33	22.2	4.72	3.49[ab]	0.82	1.61	0.80[ab]
	40PH	1.30	22.7	5.24	3.54[ab]	0.67	1.53	0.74[b]
	40FV+40PH	1.29	21.7	5.52	3.46[b]	0.71	1.57	0.70[b]
Schwarzmann	C	1.39[ab]	24.0[a]	5.01	3.72[a]	0.85	1.70[a]	0.89[a]
	40FV	1.36[a]	23.9[a]	4.88	3.70[a]	0.88	1.45[b]	0.80[ab]
	20FV+20PH	1.43[b]	22.9[b]	5.18	3.62[b]	0.91	1.46[b]	0.75[ab]
	40PH	1.39[ab]	23.3[ab]	4.95	3.67[ab]	0.85	1.41[b]	0.68[b]
	40FV+40PH	1.34[a]	22.8[b]	5.32	3.66[b]	0.88	1.42[b]	0.7[b]
Ramsey	C	1.36	23.5[a]	4.19[a]	3.59	0.70	1.69	0.86[ab]
	40FV	1.36	23.1[a]	5.33[b]	3.54	0.79	1.72	0.92[a]
	20FV+20PH	1.34	23.1[a]	4.90[a]	3.57	0.81	1.61	0.79[ab]
	40PH	1.36	23.1[a]	4.91[a]	3.59	0.78	1.53	0.74[bc]
	40FV+40PH	1.36	21.9[b]	5.01[ab]	3.58	0.77	1.50	0.64[c]

and there was no effect on berry size at harvest for the vines growing on the other rootstocks (Tab. 2). Nitrogen supply effects on berry maturity were significant for vines on 2 of the 3 rootstocks: postharvest N supply was associated with lower TSS for vines on 'Schwarzmann', and high N supply was associated with lower TSS for vines on 'Ramsey'. Nitrogen supply tends to increase the levels of titratable acidity in juice of berries from vines on 'Teleki' and 'Schwarzmann' at the highest N regime, and the supply of 40 kg N·ha^{-1} during the flowering to veraison period to vines on 'Ramsey' was associated with higher levels of titratable acidity in the juice at harvest. Despite the lack of an impact on titratable acidity, partitioning of the sums of squares indicated that juice pH of berries from vines on 'Teleki' and 'Schwarzmann' tended to be lower if the vines had been supplied with N. Specifically, the supply of 40 kg N·ha^{-1} during the flowering to veraison period to vines on 'Teleki' was associated with ca. 0.1 units lower pH compared to the vines not supplied N, and the pH of juice from vines on 'Schwarzmann' was significantly lowered by N supply during the postharvest period. The concentration of K^+ in the juice was not affected by vineyard N supply.

High levels of postharvest N supply were associated with lower levels of anthocyanins in the juice at harvest for vines on all 3 rootstocks. Nitrogen supply only decreased the levels of total phenolics in the juice of grapes from vines on 'Schwarzmann'.

Berry and juice N: Whole berry % N was increased by N supply in grapes produced by vines on 'Schwarzmann' and 'Ramsey', this increase was less pronounced by 'Teleki' (Tab. 3). In addition, berries from vines on 'Schwarzmann' and 'Ramsey' had higher % N if the vines had been supplied N during the flowering to veraison period.

Juice total N at harvest also strongly reflected N supply during the flowering to veraison period, and, as with petiole % N at veraison, there was some indication that juice N at harvest of vines on 'Schwarzmann' were influenced by N supply during the previous postharvest period. There was also some indication that the amount of N supplied during

Table 3

Effect of N supply on 'Shiraz' berry juice N pools. Values presented are means (n = 4 for 'Teleki' and n = 5 for 'Schwarzmann' and 'Ramsey'). Within columns and rootstocks means followed by the same superscript are not significantly different at P = 0.05

Rootstock	Treatment	Berry % N	mg total N/L juice	mg free amino N/L juice	mg free assimilable amino N/L	mg free non-assimilable amino N/L
Teleki 5C	C	0.086	282^{a}	53	23	30
	40FV	0.111	405^{b}	71	32	39
	20FV+20PH	0.095	308^{ab}	76	40	36
	40PH	0.102	297^{a}	50	23	27
	40FV+40PH	0.105	331^{ab}	70	34	36
Schwarzmann	C	0.086^{a}	250^{a}	66^{a}	25^{a}	41^{a}
	40FV	0.104^{bc}	414^{c}	97^{b}	46^{a}	50^{a}
	20FV+20PH	0.102^{bc}	342^{b}	85^{ab}	38^{a}	48^{a}
	40PH	0.098^{ab}	331^{b}	99^{b}	44^{a}	56^{a}
	40FV+40PH	0.114^{c}	442^{c}	153^{c}	77^{b}	76^{b}
Ramsey	C	0.089^{a}	345^{a}	69^{a}	32^{a}	37^{a}
	40FV	0.106^{b}	481^{b}	123^{b}	65^{b}	58^{b}
	20FV+20PH	0.107^{b}	360^{a}	122^{b}	66^{b}	56^{b}
	40PH	0.095^{a}	333^{a}	81^{a}	38^{a}	43^{ab}
	40FV+40PH	0.113^{b}	442^{b}	125^{b}	65^{b}	60^{b}

the flowering to veraison period affected the extent of the increase in juice N for vines on 'Schwarzmann', being less pronounced for vines on 'Ramsey' or 'Teleki'. There were only minor effects of N supply on the levels of N associated with free amino acids, assimilable amino acids and non-assimilable amino acids in the juice of vines on 'Teleki'. The influence of N supply on the levels of N associated with free amino acids in the juice of vines on 'Schwarzmann' and 'Ramsey' was more or less similar to the trend observed for juice total N. On the other hand, the amount of assimilable and non-assimilable amino N in the juice of vines on 'Schwarzmann' was only influenced by high N supply. The level of juice assimilable amino N of vines on 'Ramsey' was affected by N supply during the flowering to veraison period, irrespective of the amount. The same trend was also apparent for non-assimilable amino N in the juice of vines on 'Ramsey'.

Fermentation rate: Mean concentrations of refractable soluble solids in one of the most active ferments ('Ramsey'/40 FV + 40 PH) and one of the least active ferments ('Teleki 5C'), as measured by a refractometer, are presented in Fig. 2. The rate of soluble solids depletion was linear in all ferments for the first 7 d. The rate of soluble solids depletion in less active ferments was linear out to 10 d. There was a significant interaction between rootstock and vineyard N supply on both the rate of soluble solids depletion over the first 7 d of fermentation and the number of h taken to reach 50 % soluble solids depletion (Tab. 4). The rate of soluble solids depletion was enhanced by supply of N in the vineyard during the flowering to veraison period for musts from vines on all rootstocks, and there was evidence of a response to the amount of N supplied during the flowering to veraison period in the musts from vines on 'Ramsey'. In this regard, musts from vines on 'Schwarzmann' and 'Ramsey' also derived some benefit from N supply during the previous autumn, but the effects

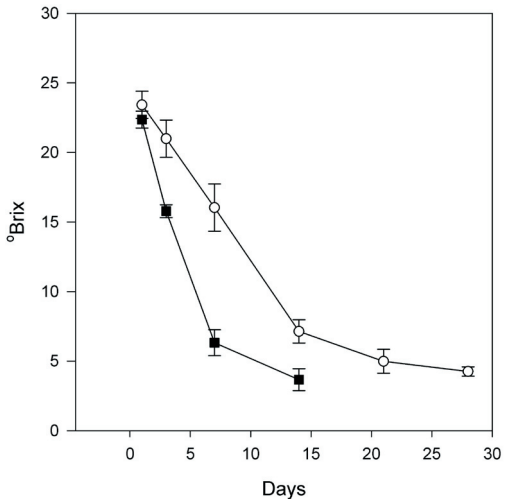

Fig. 2: TSS in fermenting musts prepared from 'Shiraz' grapes produced by 'Shiraz' vines growing on 'Teleki' (○) or 'Ramsey' (■) rootstock receiving no N fertiliser (C) or 80 kg N·ha^{-1}/season (40 FV + 40 PH), respectively. Values presented are means (n = 5) of the winemaking replicates. Vertical bars represent the standard deviation for each mean.

were less than those observed for N supplied during the flowering to veraison period. The effects of rootstock and vineyard N supply on the rate of depletion of soluble solids was directly reflected in the amount of time it took for each ferment to consume 50 % of those soluble solids. The longest times to 50 % depletion were observed in those ferments with the lowest rate of soluble solids consumption over the first 7 d of fermentation, and the shortest times to 50 % depletion were observed in those ferments with the greatest rate of soluble solids consumption.

Final wine N and residual sugar: The concentration of total N in the wine at bottling was generally increased by N supply in the vineyard (Tab. 5). In wine

Table 4

Effect of vineyard N supply on the depletion rate of refractible soluble solids over the 1st 7 d of fermentation and the time to 50 % refractable solids depletion. Within responses means followed by the same superscript are not significantly different at P = 0.05. Values presented are means (n = 5) of winemaking replicates drawn from pooled grape parcels for each rootstock × vineyard N supply treatment combination

Rootstock	Treatment				
	C	40 FV	20 FV + 20 PH	40 PH	40 FV + 40 PH
°Brix·24 h^{-1}					
Teleki 5C	1.0^{a}	1.4^{c-e}	1.3^{bc}	1.1^{ab}	1.4^{c-e}
Schwarzmann	1.4^{b-d}	1.6^{d-f}	1.4^{c-e}	1.6^{ef}	2.0^{hi}
Ramsey	1.8^{fg}	2.2^{ij}	1.9^{gh}	1.8^{fg}	2.3^{j}
h to 50 % °Brix depletion					
Teleki 5C	250^{a}	200^{cd}	214^{b-d}	239^{ab}	191de
Schwarzmann	224^{a-c}	193^{d}	208^{cd}	187^{d-f}	140^{gh}
Ramsey	162^{fg}	135^{h}	152^{gh}	166^{e-g}	140^{gh}

Table 5

Effect of rootstock and vineyard N supply on 'Shiraz' wine composition. Within responses means followed by the same superscript are not significantly different at P = 0.05. Values presented are means (n = 5) of winemaking replicates drawn from pooled grape parcels for each rootstock × vineyard N supply treatment combination

Rootstock	Treatment				
	C	40 FV	20 FV + 20 PH	40 PH	40 FV + 40 PH
mg residual N L^{-1} wine					
Teleki 5C	13^{a}	17^{ab}	33^{cd}	16^{ab}	25^{bc}
Schwarzmann	32^{cd}	64^{fg}	26^{bc}	51^{ef}	103^{h}
Ramsey	20^{ab}	64^{fg}	44de	55^{e-g}	73^{g}
g residual sugar (glucose + fructose) L^{-1} wine					
Teleki 5C	1.7^{b}	0.95^{d-g}	0.82^{e-h}	1.4^{b-d}	0.61^{f-j}
Schwarzmann	2.7^{a}	1.6^{bc}	1.1^{c-f}	1.2^{c-e}	0.30^{h-j}
Ramsey	0.61^{f-j}	0.26^{ij}	0.48^{g-j}	0.73^{e-i}	0.19^{j}

fermented from grapes produced by vines on 'Ramsey' and 'Schwarzmann' total N concentrations varied by a factor of 3 or more between the C treatment and the 40 FV + 40 PH treatment. The increase in N in the wines fermented from grapes produced by vines on 'Ramsey' and 'Schwarzmann' was due to the fact of supply, irrespective of timing or amount. The increase in residual N in the wines fermented from grapes produced by vines on 'Teleki' was much less and only present if N was applied in both periods.

Generally, wine fermented from grapes produced by vines that received N in the vineyard had significantly lower levels of residual sugar at the bottling stage than wines made from grapes from vines that didn't receive N in the vineyard (Tab. 5). Nitrogen supply during the flowering to veraison period was associated with lower levels of residual sugar, and there was some indication of a response to N supply rate on a seasonal basis across all wines. Postharvest N supply only affected residual sugar levels in wines made from grapes produced by vines on 'Schwarzmann'.

Relationships between vine indicators of N status, berry composition, fermentation behaviour and final wine composition: To identify useful predictors of fermentation performance (as affected by must N), and suitable yardsticks for those predictors, relationships between indicators of vine N status, berry N, fermentation performance and some related final wine quality parameters were explored using the means of the field replicates and the means of the wine making replicates. Significant Pearson product moment correlation co-efficients are presented in Tab. 6.

Measures of vine N status at veraison and berry juice N pools were positively related to the rate of consumption of soluble solids over the first 7 d of fermentation and negatively to the amount of time taken to deplete 50 % of the soluble solids. Particularly noteworthy are the correlations between petiole % N at veraison on the one hand and soluble solids consumption rate (Fig. 3) and the time to 50 % TSS depletion on the other. TSS consumption rate and the amount of time taken to deplete 50 % of the soluble solids also correlated significantly with % N in the lamina at veraison and must amino N and assimilable amino N at harvest. Compared to the strength of the relationships between veraison vine N status and fermentation behaviour, measurements of vine N status at flowering were less strongly related to fermentation behaviour.

The concentration of residual sugar in the final wine was negatively correlated to the measurements of vine N

Table 6

Significant (P = 0.05; n = 15) product moment correlation co-efficients for flowering, veraison, harvest, fermentation and final wine measurements. Veraison and berry composition values were the means calculated from the field replicates and the fermentation and final wine values were the means for the wine making replicates

		Flowering		Veraison			Harvest				Fermentation		Final wine	
		Petiole % N	Laminae % N	Petiole % N	Laminae % N	TSS	Berry N	Must N	Must amino N	Must assim. N	TSS consump.	H 50 % TSS	Residual N	Residual sugar
Flowering	Petiole % N	1												
	Laminae % N	0.55	1											
Veraison	Petiole % N	-	0.60	1										
	Laminae % N	-	0.62	0.90	1									
Harvest	TSS	-0.58	-	-	-	1								
	Berry N	-	-	0.66	0.70	-	1							
	Must N	-	-	0.85	0.82	-	0.78	1						
	Must amino N	-	-	0.90	0.74	-	0.67	0.77	1					
	Must assim. N	-	-	0.93	0.80	-	0.70	0.79	0.99	1				
Fermentation	TSS consump.	-	0.67	0.94	0.87	-	0.55	0.80	0.85	0.86	1			
	H 50 % TSS	-	-0.69	-0.91	-0.84	-	-0.56	-0.78	-0.83	-0.85	-0.97	1		
Final wine	Residual N	-	-	0.82	0.62	-	0.54	0.68	0.90	0.86	0.77	-0.73	1	
	Residual sugar	-	-0.62	-0.76	-0.77	0.62	-0.64	-0.69	-0.61	-0.69	-0.71	0.79	-	1

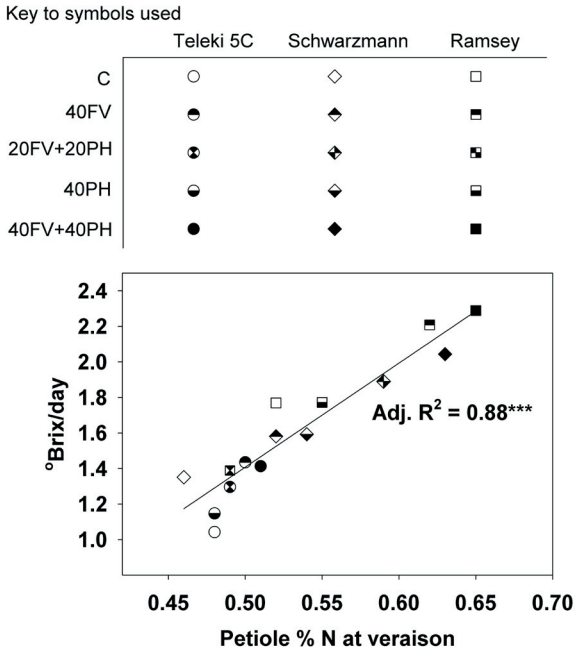

Fig. 3: Relationships between petiole % N at veraison and the daily depletion rate of refractible soluble solids over the 1st 7 d of fermentation. Values plotted are N supply treatment means (n = 5 for 'Schwarzmann' and 'Ramsey', and n = 4 for 'Teleki 5C') versus winemaking replicates (n = 5). Fitted line: g soluble solids·100 g^{-1} fermenting must.day = -1.52 [0.31] + 5.85 [0.58] (% N), adjusted r^2 = 0.88, p ≤ 0.0001. Values in square parentheses represent the standard errors for the preceding regression co-efficients.

status at veraison, and berry % N and must N pools. Residual sugar was positively correlated to the amount of time taken to deplete 50 % of the soluble solids. Residual N concentrations were positively related to veraison leaf N, and berry and must N.

Discussion

Collectively, much effort has been invested in understanding the N needs of yeast as those needs relate to vinification logistics and to final wine composition. As a result, the linkage between berry N, particularly assimilable amino N, and fermentation behaviour is well established (see Bell and Henschke 2005, and references therein). One outcome of that understanding is the identification of minimum amounts of assimilable amino N required to be in the must (Agenbach 1977) for yeast to sustain a fermentation through to dryness. Vineyard N supply strategies to manage berry N are only understood at a qualitative level, and this is reflected in the lack of suitable vine-based standards by which to assess those strategies in relation to must fermentation performance. The development of predictive tools to assist oenologists at the point of grape receival has received more attention (e.g. Dukes and Butzke 1998) than the development of vineyard-based tools to assist oenologists to predict the likely fermentation behaviour of a parcel of grapes before harvest or to assist viticulturalists to assess vineyard N management programs at developmental stages other than flowering.

In the trial reported here we manipulated N amount and time of supply to 'Shiraz' vines growing on 3 different rootstocks and measured the subsequent impact on fermentation performance as indicated by the consumption of refractable soluble solids. We related that performance back to measurements of vine N status and to various indices of berry N status at the outset of fermentation. We found a strong correlation between petiole N at veraison and subsequent fermentation performance. Soluble solids consumption rates of approximately 1.8 g·100 g^{-1} must·24 h^{-1} (*i.e.* 1 Baumé·24 h^{-1}) are considered acceptable by oenologists and closely match sugar consumption rates in synthetic grape musts over multiple yeast strains under controlled conditions (Taillandier *et al.* 2007). Using that value as a benchmark and the linear regression analysis, petiole N at veraison of greater than 0.57 % would indicate parcels of 'Shiraz' grapes that may ferment above the rate considered acceptable, and petiole N at veraison of less than 0.57 % would indicate parcels of grapes that may require supplementation to achieve a satisfactory rate of fermentation.

Supplementation of musts with DAP to bring the level of yeast available N to satisfactory levels is often made without reference to the amount of assimilable amino N

already present in the must. Given the link between yeast available N (including ammonium), urea in the wine at the end of fermentation, and the subsequent formation of ethyl carbamate from the reaction of ethanol and urea (Ough *et al.* 1989), indiscriminant use of DAP carries risk. The suboptimal addition of DAP leads to high residual N and this can result into microbiological instability (Bell and Henschke 2005). Such a usage pattern may stem from the logistical difficulties associated with estimating yeast available N at the point of receival. We have previously shown that across seasons veraison leaf tissue N is a reasonable predictor of assimilable amino N at harvest (Holzapfel and Treeby 2007), and here we have presented data showing that fermentation behaviour is strongly related to leaf tissue N at veraison, as well as assimilable amino N. That relationship could be used to allow timely and more precise prediction of the fermentation management strategies that may need to be deployed for a particular parcel of grapes. That relationship may also allow viticulturists to assess vineyard N supply management programs against a target tissue level at a defined developmental stage.

Using grapes produced by 'Shiraz' vines growing on 3 different rootstock genotypes that had consistently received the same N fertiliser treatments for 3 seasons, indices of must fermentation behaviour were related to vine N status at veraison and berry N status at harvest. A strong ($R = 0.94$) correlation was established between leaf petiole % N at veraison and consumption of soluble solids over the first 7 d of fermentation. Fermentation rate was also related to assimilable amino N and total free amino N pools in the berries at harvest, but the relationships were not as robust. Industry norms regarding fermentation allow identification of a veraison petiole N concentration that may be used as a standard. Veraison petiole % N potentially offers viticulturalists another time point within a growing season to assess vineyard N supply strategies, and offers oenologists a timely predictive tool to manage fermentation of parcels of grapes.

Acknowledgements

We thank P. Smith and the Sunraysia Nurseries, Gol Gol, NSW for the use of the vineyard and their assistance in conducting the field trial. In addition we would like to thank the AWRI staff who conducted the wine residual sugar determinations. Mr. S. Warne, Casella Wines, Griffith, NSW, is thanked for helpful discussions during the preparation of the manuscript.

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Received September 12, 2012