

Effects of timing of N application and water constraints on N accumulation and juice amino N concentration in 'Chardonnay' grapevines

B. P. HOLZAPFEL¹, J. WATT², J. P. SMITH², K. ŠUKLJE² and S. Y. ROGIERS¹

¹National Wine & Grape Industry Centre, NSW Department of Primary Industries, Wagga Wagga, Australia

²National Wine & Grape Industry Centre, Charles Sturt University, Wagga Wagga, Australia

Summary

The amount and timing of nitrogen (N) application to a vineyard is critical for must yeast assimilable nitrogen (YAN) concentrations. YAN concentrations and amino acid profiles are important for the fermentation process and wine composition. Commonly, N is applied at flowering to optimize leaf functioning or after harvest to enhance vine productivity the following season. In this study N was applied at various stages of berry development to determine allocation patterns between vine perennial and annual components and to assess when berry YAN concentrations can best be optimized. Five year old potted 'Chardonnay' vines received ammonium sulfate fertilizer at six different times from full bloom to two weeks before harvest and were also exposed to either full or half irrigation during that period. Reduced water supply resulted in a higher allocation of N to the perennial structures and less to the annual components of the vine. N allocation to the annual components of the vine was greatest when it was applied at full bloom, however allocation to the perennial components was greatest when it was applied after fruit-set to veraison. The timing of N supply had a substantial influence on YAN concentrations, and was highest when N was applied about two weeks after veraison. Low water supply also resulted in higher juice YAN concentrations. The perennial N reserves in the roots were highest under low water supply and when N was applied at veraison, while the allocation to the annual parts was lower under this irrigation regime. The study indicates that timing of N application and the application of water constraints during berry development can impact on N partitioning, while the total amount accumulated by the vine is not altered.

Key words: nitrogen supply; water status; partitioning; amino acids; 'Chardonnay'.

Introduction

The uptake, storage and mobilization of nitrogen (N) are important processes within perennial crops like the grapevine. The accumulation of N into the storage sinks

of the perennial structures prior to harvest is important for vine development the following season, when root uptake is limited in early spring and also when water supply is low during the season. The N requirements for annual growth and development are therefore met through acquisition from the soil and mobilization from the perennial storage tissues. The relative contributions from these two processes vary during the season, with N transfer from annual organs also occurring simultaneously. The amount of N allocated to the berry is critical for the yeast assimilable nitrogen (YAN) concentration in the must at harvest, and therefore for the wine making process and the final wine composition.

Nutrient uptake varies during the growing season, important N uptake periods occur between bloom and veraison and after harvest, when most of the root growth commonly takes place (CONRADIE 1980). Before flowering, shoot growth is strongly dependent on N mobilized and transported from reserves stored during the previous season (ZAPATA *et al.* 2004). The leaves, shoots and bunches are strong sinks for N assimilated during the period from flowering to veraison (CONRADIE 1991). Between veraison and harvest, when N uptake might slow or cease altogether, redistribution from roots, shoots and leaves to bunches takes place (CONRADIE 1991), with the majority of the N accumulated into berries after veraison (SCHALLER *et al.* 1990). The redistribution of N is, however, dependent on N availability during the berry ripening period; impacting on the temporary (berries) and storage sinks (wood and roots). The decline in N reserves in the perennial structure leads to further uptake of N and other nutrients during the post-harvest period (BATES *et al.* 2002). The quantities of N winter reserves vary due to cropping levels (BALASUBRAHMANYAM *et al.* 1978), N supply (TREEBY and WEATLEY 2006), pruning systems (RUHL and CLINGELEFFER 1993) and environmental conditions, ranging between 10 and 75 g N per vine (LÖHNERTZ *et al.* 1989; TREEBY and WEATLEY 2006). About 30 % of the stored N used in the following season's development derived from N accumulated in the perennial structure (CONRADIE 2005), being particularly important for canopy growth in early spring.

Aside from seasonal influences, the main drivers of N uptake are the amount of water and N fertilizer applied to a vineyard. The timing of N application is also critical for influencing vine growth and yield and the proportion of

Correspondence to: Dr. B. P. HOLZAPFEL, National Wine & Grape Industry Centre, NSW Department of Primary Industries, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. Fax: +61 02 6933 4068. E-mail: bruno.holzapfel@dpi.nsw.gov.au

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N directed towards the berries. N applied at bud burst is rapidly incorporated into the vegetative tissues, while an application at bloom can increase yield (LINSENMEIER *et al.* 2008) and also berry YAN concentrations (NEILSEN *et al.* 2010). N supplied over the bloom and veraison period results in higher berry N compared to an application in the post-harvest period, with the later stimulating early canopy development and yield in the following season (HOLZAPFEL and TREEBY 2007). A foliar application of N can elevate YAN considerably if applied around veraison (HANNAM *et al.* 2014) or several weeks after (LASA *et al.* 2012). Seasonal water deficit limits the uptake of N (KELLER 2005), reducing vegetative growth and yield as well as elevating proline concentration and other amino N in the must (MATTHEWS *et al.* 1987; MATTHEWS and ANDERSON 1988, 1989; HANNAM *et al.* 2013). In contrast, reduced water supply in the form of partial rootzone drying (PRD) or regulated deficit irrigation (RDI) can lower berry proline and arginine N concentration (WADE *et al.* 2004). The concentrations and composition of nitrogenous compounds in the must are of major importance for the wine making process.

YAN in the must is composed of free assimilable amino N (FAN) and ammonium, with the minimum requirements for yeast being about 130 mg YAN per L of must for the completion of fermentation (AGENBACH 1977). Musts with low concentrations of YAN are associated with sluggish and stuck ferments (INGLEDEW and KUNKEE 1985), while musts with high levels ferment too fast (MONTEIRO and BISSON 1991). In addition to the amount of YAN in the must, low N results in elevated levels of undesirable thiols and higher alcohols and lower concentrations of esters and long chain volatile fatty acids in the wine. In contrast, high N must leads to an increase in ethyl acetate and acetic acid, ethyl carbamate and biogenic amines in the wine (BELL and HENSCHKE 2005). Arginine and proline are usually the dominating amino acids, with N from the later not assimilated by the yeast during the fermentation process. The composition of the individual amino acids in the must is also important for wine composition, since the various amino acids provide C-skeletons for certain flavor compounds (BELL and HENSCHKE 2005). Insufficient must YAN can be enhanced by adding di-ammonium-orthophosphate (DAP) in the winery. However, since the amino acid composition and amounts are crucial for wine flavor, it is best to use DAP only for fine tuning the must, while the grape amino-N concentration should be optimized in the vineyard.

This research was undertaken to investigate the timing of N supply and the effect of water constraints on vegetative and reproductive development and the allocation of N to the perennial and annual components of the vine. The aim was to provide a better understanding for the timing of N application on juice nitrogenous compounds, particularly YAN and on storage N of the perennial structure.

Material and Methods

Plant material and treatments: The pot experiment was conducted in an outside bird-proof enclo-

sure located at the National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, New South Wales (Australia). The five year-old own rooted 'Chardonnay' (Clone I10V5) vines were planted in 50L pots and were filled with a commercial potting mix which allowed for a water holding capacity of approximately 8 L of plant available water per pot. The top of the pots around the vine trunks were covered with plastic, to avoid rain water entering the soil. There were four rows with 12 vines, with distance between vines 1m and between rows 1.4 m. Vines were trained to a single cordon (at 40 cm above the pots) and thinned to 12 shoots and one bunch per shoot (wherever possible). The shoots were vertically trained and fixed to foliage wire (at 105 cm above the pots). The vines were sprayed with preventive copper and sulfur sprays during the growing season to prevent mite and fungus infections. Shortly before bloom (beginning of flowering occurred on November 10) and prior to the treatment implementations all vines received an initial 2 g dose of N (3/11/2010) as ammonium sulfate, with N-free Megamix Plus® (Rutec, Tamworth, Australia).

There were four replicates of each of the six N timing applications (N1, N2, N3, N4, N5 and N6) and two water regimes (FI and HI), resulting in 12 treatment combinations, in a fully randomized block design. The water supply regimes were implemented from full bloom on November 18 (2010) to harvest February 9 (2011) and consisted of full irrigation (FI) and half irrigation (HI) using 4 L and 2 L drippers respectively. The soil moisture was monitored with TDR probes placed in every second pot (Trace, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA). These measurements were conducted twice a week early in the morning and late afternoon, to assess irrigation requirements. The volumetric water content of the soil being about 4 % lower under half irrigation compared to full irrigation, with the average values being 6.7 and 10.8 % respectively. The vines received 4 g of N at each of the six application times. The first N treatment was applied on November 17 (2010), corresponding to 70 d after budburst (September 8), and the last of the six fortnightly applications occurred on January 26 (2011), two weeks before harvest. Therefore the first four N applications (N1 – N4) were applied before veraison (December 29) and the last two (N5, N6) during berry ripening. Every 'Chardonnay' vine received 4 g of N as dissolved ammonium sulfate (19.05 g in 100 mL). The total amount (pre treatment and treatment application) was based on a 'Chardonnay' field study where each shoot contained about 1.0 g N at harvest (with 25 % already accumulated at flowering and about 25 % in each of the bunches of a shoot). To maintain the nutrient status for the other minerals, 5 mL diluted N-free Megamix Plus® was also applied every two weeks (therefore six times) between the N applications, starting on November 24 (2010), with the last one applied a week before harvest (2 February 2011).

Measurements and sampling: The response of the two water supply regimes and the six timings of N supply on leaf chlorophyll was assessed with a Spad meter (Spad-502, Minolta Corp. Ltd, Osaka, Japan) and on pre-dawn leaf water potential Ψ_{pd} by pressure bomb (3000,

Soil Moisture Equipment Corp. Santa Barbara, CA, USA) during the treatment implementation. The chlorophyll index was determined on the six leaves along one shoot starting from the remaining basal bunch, the measurement for a leaf consisted of an average of three readings. The Ψ_{pd} was assessed on a fully unfolded leaf for each vine in weekly intervals to determine vine water status and to provide further information for irrigation requirements. For clarity the chlorophyll data are only presented in response to the N treatments and the Ψ_{pd} for the irrigation treatments.

The vines were harvested at about 20° Brix on February 9 (2011). Prior to harvest, six berries were sampled for amino-N determination (one berry from six bunches per vine) and were then juiced and stored at -80 °C until analysis. Prior to freezing the samples, total soluble solids (TSS) were determined with a digital bench refractometer (PR-101, Atago, Tokyo, Japan). Sugar per berry was calculated by multiplying per cent TSS with berry weight, similarly sugar per vine by multiplying per cent TSS with yield. Total grape yield per vine was recorded, the berries were removed from the bunchstem and the fresh weight of berries per cluster and bunchstem was recorded prior to drying. The final shoot length at harvest was measured from six shoots per vine, and then the vines were separated into various annual and perennial vegetative parts to assess the dry matter and N accumulation shortly after harvest. This destructive harvest of the vegetative parts was undertaken from February 14 to 17, with one replicate of 12 vines processed per day and the last root fresh weight was determined on February 18, 2011. All 12 shoots were separated into leaves and stems, while the perennial parts of the vines were destructively harvested and divided into five sections. These included the cordon arms with spurs, the cordon section separated from the trunk below the lowest cordon arm, the trunk cut at soil level, and the remaining rootstock separated from the roots. For reporting, N amounts of the cordon arm and cordon were later combined for simplification. The fresh weight of all vegetative parts was determined prior to washing, except the roots and the rootstock which were weighed after excess soil was removed through washing and air drying for about 18 h. All parts were washed with a phosphate free detergent and then rinsed twice with distilled water. The various parts were placed into a drying oven at 60 °C, with dry weight recorded after completion of the drying process. The time for drying varied between the different tissues, from a few days for leaves to several weeks for berries and was determined when no further weight loss was observed.

The dried samples were first ground to 0.5 mm using a heavy duty cutting mill (Retsch ZM2000, Retch GmbH, Haan, Germany), then sub-samples were taken from all dried tissues and ground through a 0.12 mm sieve (Retsch ZM100) for analysis for total N content. The total N content was determined using a Carlo Erba CE1110 CHN-S analyzer by the ANU analytical services (Milan, Italy). The amino-N was determined by using an HPLC method for amino acids (HYNES *et al.* 1991). A sub-sample of juice of six berries was filtered (45 μ m) with micro-spin filter tubes (Alltech Associates Inc., IL, USA) and diluted 1:20 with 0.25 M borate buffer (pH 8.5). The amino groups were

derivatised with 9-fluorenyl-ethyl chloroformate prior to separation with a reverse phase column (Thermo 5 μ m ODS hypersil, Waltham, MA, USA) and quantified with an HPLC equipped with a fluorescence detector (Waters 2475, Milford, MA, USA). Ammonium concentrations in the juice samples were assessed after centrifuging using an enzymatic kit (Megazyme International, Bray, Ireland), then determined colorimetrically at 340 nm with a spectrophotometer (UV-1700 PharmaSpec, Shimadzu Cooperation, Kyoto, Japan).

The N concentration of free amino acids, expressed in mg·L⁻¹ related to the α amino N atoms (except for proline). The total free assimilable amino N (FAN) concentrations were calculated from the α amino N atoms of 17 free amino acids, with the exception for arginine were two N atoms were used. The total yeast assimilable N (YAN) was calculated from the addition of FAN and the N atoms of ammonium. The non-assimilable amino N (NAN) was calculated from the number of non- α amino N atoms of each amino acid (HOLZAPFEL and TREEBY 2007).

Data analysis: The data were compiled in Excel 2003 and further analyzed using the graphic program Sigma plot version 11.0 (Systat Software Inc., San Jose, California, USA). Individual data points in the graphs are shown with error bars representing ± 1 standard error of the mean ($n = 12$ for irrigation treatments, $n = 8$ for N timing treatments). Data analysis was conducted using the statistical program Genstat version 16.1 (Rothamsted Experimental Station, Harpenden, Herts, United Kingdom). Analysis of variance was conducted ($p < 0.05$, 0.01 and 0.001) and Fisher's LSD test was used to identify significant differences between means ($p < 0.05$) for the harvest data, the amino acid concentrations and the N amounts of the various vine parts.

Results and Discussion

The vines responded to the N and irrigation treatments with differences in vegetative and reproductive growth and development. At harvest the amounts of N allocated to the various sections of the vine was influenced by both water supply and the time of N application, with the amount and concentrations of the nitrogenous compounds in the juice also altered.

Physiological responses: The impact of the two irrigation treatments were observed in the more negative pre-dawn leaf water potential (Ψ_{pd}) shortly after implementation and became more pronounced after about a month, with the HI being about 0.2 MPa lower than the FI (Fig. 1A). The differences in Ψ_{pd} between FI and HI was less pronounced closer to harvest, with -0.23 and -0.28 MPa, respectively. Those vines that received half the water of the fully irrigated vines did undergo a reasonable amount of water constraint at around the onset of ripening (CARBONNEAU *et al.* 2004).

The N treatments did not alter the Ψ_{pd} , except for the N1 treatment which resulted in higher values than most other treatments on one occasion (one week after treatment implementation). The chlorophyll index increased after

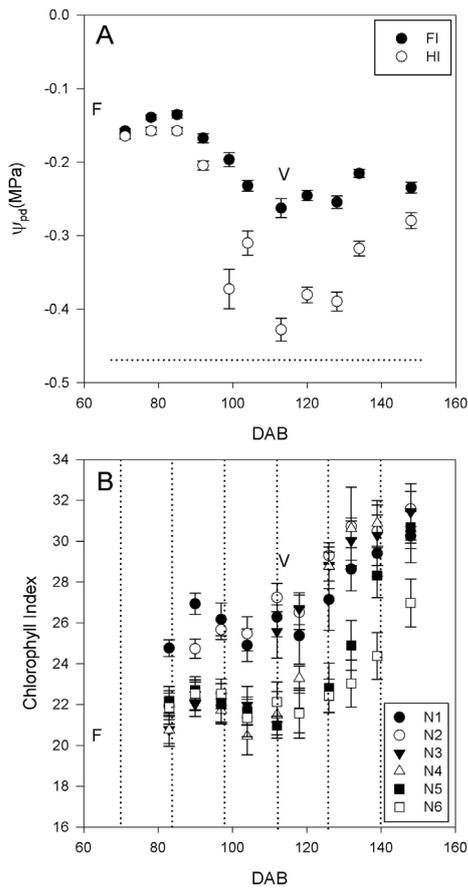


Fig. 1: The responses of water supply (A: half irrigation and full irrigation) and timing of N application (B: full bloom to harvest) on leaf water potential and chlorophyll index from full bloom to harvest (DAB = days after budburst), the water supply and application of N are indicated with dotted lines (F = Flowering, V = veraison). Error bars ± 1 SE mean.

each N application, from flowering towards harvest, from 20 to 25 and 27 to 32 units respectively (Fig. 1B). The first application in the middle of November (N1) resulted in a 2 to 4 unit increase within two weeks. The largest increase was observed after the application of N prior to veraison (N4), with an increase of nearly 10 units in three weeks. It appeared that the application from veraison and thereafter resulted in the most significant elevation of chlorophyll content of the leaves, which could indicate that more N is allocated to the annual organs from uptake or re-mobilization from the storage tissues. An additional increase after veraison of the chlorophyll index was also present for the earlier N application timings (N1 to N3), which could be partially due to an accumulation of other nutrients such as magnesium and zinc (POTARZYCKI 2011) supplied between the N applications. However, it has been observed that clusters after veraison can attract newly absorbed N and/or N from the perennial structure (CONRADIE 1980, 1991), which indicated that the annual organs act as a stronger N sink than the perennial parts during berry ripening. The irrigation also influenced the chlorophyll index and the HI treatment was usually about two units lower than the FI treatment throughout berry development. This was also reflected in leaf N content at harvest (Tab. 2), suggesting a reduced uptake in the HI treatment and an influence on

the N translocation. N uptake under limited water supply is often reduced, leading to reduced growth and N tissue concentrations (ALLEWELDT *et al.* 1984). However, these findings indicate that vines responded to each additional N application to the initial rate, regardless of the time during the season. This suggests an increased N incorporation into the leaves after each time the N was applied (N1 to N6) There were no differences apparent in leaf N at harvest (Table 2), indicating the final total allocation to the leaves was independent of the timing of application.

Shoot length and yield parameters: Shoot length was reduced in those vines which were supplied with less water (Fig. 2). Almost immediately after

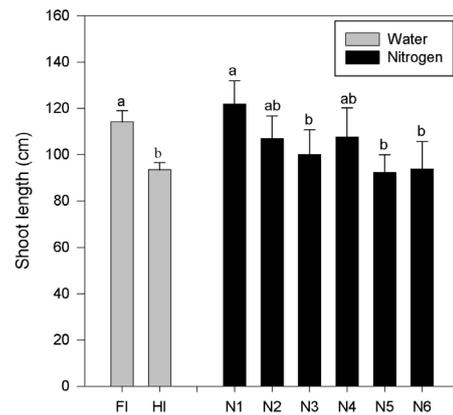


Fig. 2: Shoot length at harvest in response to water supply (half irrigation and full irrigation) and timing of N application (full bloom to harvest). Means separated within columns using Fisher's LSD test. Different letters indicate a significant difference ($p = 0.05$). Error bars ± 1 SE mean.

the irrigation treatments were initiated, at 71 d after budburst, the differences in shoot length between FI and HI became apparent. The differences in shoot length were maintained for the following two to three weeks, with the overall growth ceasing two weeks prior to veraison in the middle of December (data not presented). The N applied at the earliest time (N1) had the most impact on elevating shoot growth and final shoot length to 122 cm at harvest (Fig. 2). The last applications (N5 and N6) during the berry ripening phase had the shortest shoots at harvest, 92 and 94 cm respectively. As observed for the two irrigation treatments, the shoot growth ceased about two weeks prior to veraison, therefore the impact on shoot growth of the N application at the later times was minimal. A reduction in shoot growth by limited water and N supply is a common response and has been reported previously (HOLZAPFEL and TREEBY 2007; ALLEWELDT *et al.* 1984). However, the response to the timing of soil applied N throughout the berry development provides new information, by indicating the point of time when N application is less likely to enhance shoot growth and likely to contribute more to the nitrogenous compounds in the berry or to the accumulation of N in the root and wood tissues.

Berry fresh weight at harvest was significantly lower in the HI treatment at 1.15 compared with 1.24 g in the FI treatment (Tab. 1). There was no impact of the N treatments on berry fresh weight, however the earliest N application

Table 1

Yield and berry parameters influenced by water supply (half irrigation and full irrigation) and timing of N application (full bloom to harvest)

	Yield (g)	Cluster wt (g)	Berries/ cluster	Berry wt (g)	TSS (Brix)	Sugar/berry (mg)	Sugar/vine (g)
FI	1013a	84.4a ^a	68.0a	1.24a	20.4a	254a	207a
HI	775b	64.6b	56.1b	1.15b	19.8b	228b	153b
N1	1271a	105.9a	88.7a	1.20	20.8a	251	264a
N2	894b	74.5b	54.9b	1.33	20.4a	271	183b
N3	766b	63.8b	54.6b	1.16	20.3ab	236	155b
N4	780b	65.0b	56.0b	1.14	20.6a	236	161b
N5	855b	71.2b	61.6b	1.16	19.0c	221	163b
N6	798b	66.5b	56.7b	1.19	19.5bc	232	155b
Water	**b	**	*	*	*	**	***
N	**	**	**	ns	**	ns	***
Interaction	ns	ns	ns	ns	ns	ns	ns

^a Means separated within columns using Fisher's LSD test. Different letters within a column indicate a significant difference ($p = 0.05$).

^b *, **, ***, and ns indicate significance at $p \leq 0.05$, ≤ 0.01 , ≤ 0.001 , and not significant, respectively.

Table 2

N quantities in the annual vegetative and reproductive parts at harvest influenced by water supply (half irrigation and full irrigation) and timing of N application (full bloom to harvest)

	Berries	Bunchstems	N (g·vine ⁻¹)		Stems	Shoots	Annual
			Cluster	Leaves			
FI	0.97	0.042b ^a	1.01	2.20a	0.35	2.56a	3.57a
HI	1.15	0.064a	1.21	1.62b	0.37	1.99b	3.20b
N1	1.44	0.054ab	1.49	2.17	0.38	2.55	4.04a
N2	1.15	0.049b	1.20	1.88	0.35	2.23	3.43b
N3	0.82	0.047b	0.87	1.89	0.36	2.25	3.12bc
N4	0.85	0.041b	0.89	1.77	0.39	2.15	3.04c
N5	1.09	0.067b	1.16	1.91	0.37	2.27	3.43b
N6	1.00	0.064a	1.06	1.84	0.33	2.17	3.23bc
Water	ns	***b	ns	***	ns	***	*
N	ns	**	ns	ns	ns	ns	*
Interaction	ns	ns	ns	ns	ns	ns	ns

^a Means separated within columns using Fisher's LSD test. Different letters within a column indicate a significant difference ($p = 0.05$).

^b *, **, ***, and ns indicate significance at $p \leq 0.05$, ≤ 0.01 , ≤ 0.001 , and not significant, respectively.

(N1) led to the highest fruit yield (Tab. 1) and shoot length. Water stress applied after fruit set until veraison (RDI) is commonly used to reduce berry size and to enhance fruit composition in red varieties. This irrigation strategy also led to a reduction in shoot growth and pruning weights (MATTHEWS *et al.* 1987, MATTHEWS and ANDERSON 1989). An increase of N and water supply often elevates yield and shoot growth, with the timing of supply being critical to the impact on both these parameters.

The yield was reduced by about 25 % in vines that received half the water compared to the ones that received full irrigation. This reduction was due to not only reduced berry weights but also a decline in berries per bunch, being 17.5 % lower for the HI treatment. HI berry sugar concentrations expressed in total soluble solids (TSS) at harvest were also reduced and consequentially sugar per berry by 10 % and sugar per vine by 26 % (Tab. 1). These responses were due to the impact of water stress on fruit set, since the vines would have had similar flower numbers prior to the implementation of the irrigation treatment. The berries would have expanded less (reduced cell numbers and size) because of the water deficit on the berry itself and reduced assimilate production (MATTHEWS and ANDERSON 1989; PE-

REZ PEÑA and TARARA 2004). N application at full bloom led to higher yield than all the other treatments, due to higher number of berries per bunch. The application of N two and four weeks prior to harvest (N5 and N6) led to small reduction in berry sugar concentrations (TSS). The lower berry number per bunch in all the N applications after the first one suggests an impact on final berry set due to insufficient N during fertilization. The reduction in berry number due to a limited N during the flowering and fruit set period has been observed previously (KELLER *et al.* 2001). The reduced berry sugar concentrations (TSS) in response to the last two N applications could be due to a lower N status in the early part of the ripening process, which likely impacted on the photosynthetic efficiency and the assimilate production of the leaves (PONI *et al.* 1994). The chlorophyll index of the N6 treatment was significantly lower shortly before harvest, indicating a lower leaf N content and the possible impact on photosynthesis. Both treatment times (N5 and N6) were too late to have a significant impact on ripening during the rapid sugar accumulation phase. In addition, photoassimilate may have been diverted to root growth and N uptake to increase vine N status (KELLER and KOBLET 1995). Overall, N supply appears to be critical for

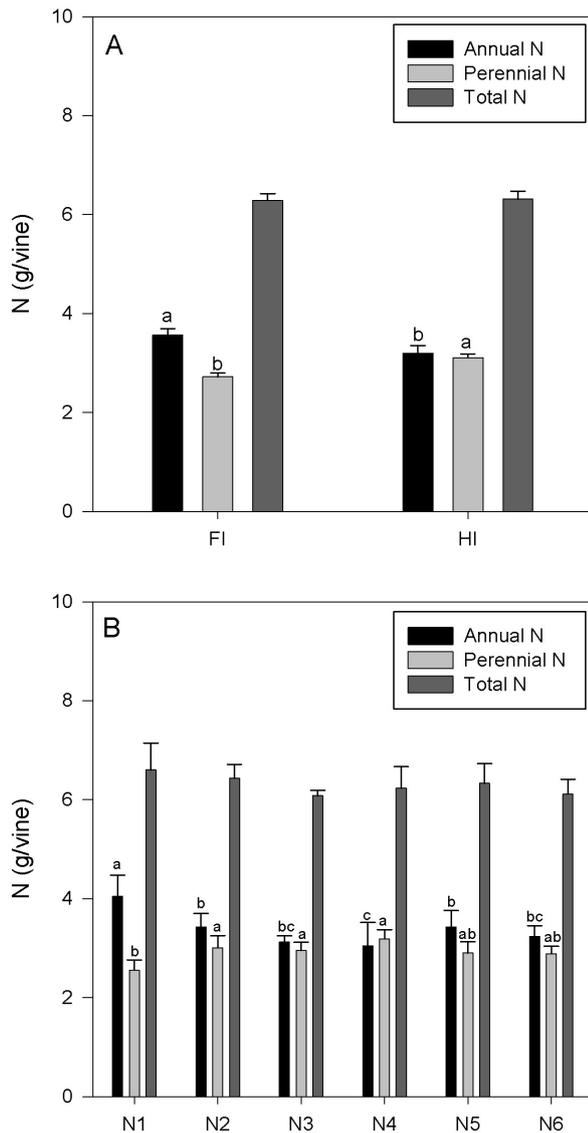


Fig. 3: Annual, perennial and total N at harvest in response to water supply (A: half irrigation and full irrigation) and timing of N application (B: full bloom to harvest). Means separated within columns using Fisher's LSD test. Different letters indicate a significant difference ($p = 0.05$). Error bars ± 1 SE mean.

fruit-set early in the season and to a lesser extent for berry sugar accumulation during the berry ripening phase.

Annual and perennial N: The leaves contained the majority of the N (two thirds) that was allocated to the annual growth; whereas the N located within the perennial components of the vine were mostly found in the root system (Tabs 2 and 3). The water supply and the timing of N application had no impact on the total accumulation of N in the vine, although both treatments altered the partitioning between the annual and perennial components (Fig. 3).

In comparison to FI, lower water supply elevated the amount of N stored in the perennial tissue while the amounts in the annual tissue were reduced. Water constraints resulted in a 25 % reduction in the total N allocated to the leaves (Tab. 2), and this was due to shorter shoots with less and smaller leaves. The leaf N content of vines exposed to only HI were elevated by about 25 % in comparison to leaves from FI vines. A concentration increase was also observed for other annual parts (shoots and bunchstems). All perennial components, in particular the parts below ground, had elevated amounts of N if the water application was reduced, except the cordon which had an insignificant elevation (Tab. 3). This suggests there is an accumulation of N in the roots under reduced water supply. This could be due to lower growth requirements in the aboveground components, which had overall less N under low water supply. Elevated N accumulation in the roots under water limitations has been observed in a previous experiment (MÜLLER *et al.* 2015) and can be explained by the more pronounced N assimilation processes by these water stressed roots. However in comparison to FI vines, low water supply reduced overall N allocation to the annual components but increased it to the perennial components (Fig. 3A).

Early N application resulted in the highest N amount in the annual organs. However, when it was applied shortly after veraison it resulted in the lowest levels in these organs (Fig. 3B). Later N applications, particularly around veraison, resulted in greater allocation of N to perennial parts, rather than annual parts. This indicates that the pri-

Table 3

N quantities in the perennial wood and roots at harvest influenced by water supply (half irrigation and full irrigation) and timing of N application (full bloom to harvest)

	N ($\text{g}\cdot\text{vine}^{-1}$)						
	Cordon	Trunk	Wood	Roots	Rootstock	Rootsystem	Perennial
FI	0.32	0.36b ^a	0.69b	1.74b	0.30b	2.03b	2.72b
HI	0.36	0.41a	0.76a	2.00a	0.35a	2.35a	3.11a
N1	0.32	0.37	0.69	1.60	0.27	1.87b	2.56b
N2	0.37	0.46	0.83	1.81	0.36	2.18ab	3.01a
N3	0.33	0.35	0.68	1.91	0.36	2.28ab	2.95a
N4	0.38	0.39	0.77	2.08	0.34	2.42a	3.19a
N5	0.34	0.37	0.71	1.86	0.33	2.19ab	2.90ab
N6	0.31	0.37	0.68	1.95	0.26	2.21ab	2.89ab
Water	ns ^b	*	*	*	*	**	***
N	ns	ns	ns	ns	ns	*	*
Interaction	ns	ns	ns	ns	ns	ns	ns

^a Means separated within columns using Fisher's LSD test. Different letters within a column indicate a significant difference ($p = 0.05$).

^b *, **, ***, and ns indicate significance at $p \leq 0.05$, ≤ 0.01 , ≤ 0.001 , and not significant, respectively.

ority of partitioning between annual and perennial organs alters during the period between fruit set and harvest. This switch could be due to different growth requirements, from shoot development to further development of the perennial structure and/or storage of N reserves.

The N concentrations within the leaves at harvest were the highest for N6 (1.79 % N) and lowest for N4 (1.61 % N) and did not undergo the same trends as the chlorophyll index, which increased after each N application. However, this is in some respects understandable, since the measurements were carried out during the period of berry development and the leaves would have accumulated more N after each treatment application. However, the significantly lower chlorophyll index of the N6 treatment (Fig. 1B) prior to the destructive harvest (10 d) was not reflected in the N concentrations of the leaves. Although the bunches tended to have an elevated N content in the two early N treatments, the only significant differences were observed in the bunchstems, which had the highest N amounts when N was applied four weeks before harvest (N5). The root system contained the highest amounts when N was applied six weeks before harvest (N4) and lowest in vines that received N at the end of flowering. This indicates that during early berry development the applied N has been allocated predominantly to the bunches and shoots. The later N application at around veraison indicates that it has not moved to the annual organs, but rather to the root system as indicated previously.

Juice amino acids and ammonium: The allocation of N towards the berries tended to be less if N was supplied from around veraison to shortly before harvest. However, from the grape composition point the must YAN levels are most critical. The YAN concentrations in the juice were elevated by 36.8 % under low water supply compared to the full water supply. These differences between the irrigation treatments were also present in the form of elevated (37.5 %) ammonium concentrations (NH₄), the assimilable amino acid N (FAN) increase of 39.2 %, as well as the non-assimilable N fraction (NAN) with an increase of 32.5 % (Fig. 4A). Similar increases in proline concentrations have been reported, when water stress was implemented after veraison (MATTHEWS and ANDERSON 1988). Pre-veraison water stress has been demonstrated to increase YAN concentrations in the berries (HANNAM *et al.* 2013). This is in contrast to other findings in a field situation, where arginine, a large part of the FAN of the juice, was lowered by water stress imposed by PRD or RDI (WADE *et al.* 2004).

The increase apparent in the current study could be due to a concentration effect, since the berries were about 10 % smaller when the irrigation was reduced by half. In addition, the water stress was implemented throughout the whole of berry development as opposed to the fruit set to veraison period (RDI) or half the rootzone (PRD), with the later not reducing berry weight (WADE *et al.* 2004). Higher amino acid N was present for most amino acids (Tab. 4) under low water supply in comparison to the high water application, except for glutamine (GLN), γ -aminobutyric acid (GABA), methionine (MET), lysine (LYS) and phenylalanine (PHE).

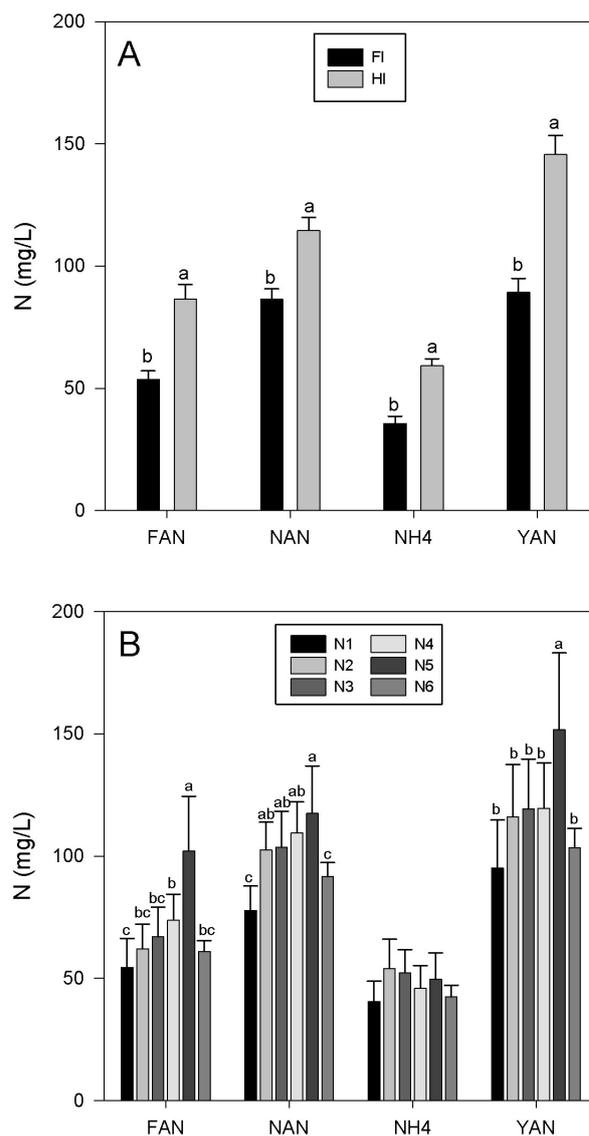


Fig. 4: The impact of water supply (A: half irrigation and full irrigation) and timing of N application (B: full bloom to harvest) on free assimilable amino N (FAN) and non-assimilable amino N (NAN), ammonium N (NH₄) and total yeast assimilable N (YAN) in the berry juice at harvest. Means separated within columns using Fisher's LSD test. Different letters indicate a significant difference ($p = 0.05$). Error bars ± 1 SE mean.

N was most effective at increasing some juice nitrogenous compounds when applied four weeks (N5) before harvest compared to earlier or later N applications. Applying N during this period resulted in about 1/3 higher concentration of NAN and YAN, (Fig. 4B). The FAN fraction was elevated by 40 % compared to the full bloom application, with the NH₄ concentrations not altered by the timing of N application. This suggests that an N application about two weeks after (N5) veraison is most effective in elevating YAN in the juice, followed by the three N application times (N2 to N4) prior to veraison, while an application at the conclusion of bloom and two weeks before harvest were the least effective. Others have demonstrated that foliar N application around veraison resulted in similar increases in YAN concentrations (HANNAM *et al.* 2013). This increase

Table 4
Juice amino acid N at harvest influenced by water supply and timing of N application (full bloom to harvest)

	Amino acid N (mg·L ⁻¹)																	
	PRO	ALA	GLN	SER	ARG	THR	GABA	PHE	VAL	HIS	ASP	LEU	GLY	ILE	ASN	TYR	MET	LYS
FI	68.5b ^a	14.0b	7.5b	8.0b	6.8b	9.3b	2.86	2.74	2.13b	1.58b	1.03b	0.87b	0.77b	0.75b	0.30b	0.27b	0.21	0.17
HI	79.9a	23.1a	13.8a	11.5a	14.9a	14.2a	3.09	2.69	2.93b	2.59a	1.28a	1.17a	1.37a	1.08a	0.53a	0.49a	0.19	0.17
N1	58.3b	13.9c	6.5c	6.7d	8.6bc	9.3c	3.86a	2.74	2.12b	2.00	0.84e	0.95	0.96bc	0.81	0.27c	0.36bc	0.11	0.14
N2	80.2a	16.2c	8.1bc	8.0c	9.7bc	11.2bc	3.02ab	3.21	2.49ab	2.07	0.99d	1.00	0.98bc	0.93	0.32bc	0.37bc	0.16	0.15
N3	79.5a	17.7bc	9.3bc	9.0bc	9.8bc	12.2bc	3.17ab	2.54	2.69a	2.23	1.06cd	1.03	1.10b	0.93	0.38bc	0.39b	1.45	0.31
N4	82.2a	20.6b	10.6bc	9.5bc	11.8b	12.9b	3.19ab	2.56	2.74a	2.18	1.19bc	1.09	1.02b	1.00	0.46b	0.41ab	0.19	0.16
N5	75.2a	27.3a	17.7a	14.2a	18.8a	15.8a	2.47bc	2.66	2.94a	2.49	1.59a	1.19	1.74a	1.06	0.67a	0.55a	0.22	0.15
N6	70.0ab	15.6c	11.6b	11.0b	6.5c	9.3c	2.15c	2.61	2.18b	1.53	1.26b	0.86	0.61c	0.77	0.38bc	0.21c	0.07	0.10
Water	*	***	***	***	***	***	ns	ns	***	***	***	***	***	***	***	***	ns	ns
N	*	***	***	***	***	***	**	ns	**	ns	***	ns	***	ns	***	**	ns	ns
Interaction	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns

^a Means separated within columns using Fisher's LSD test. Different letters within a column indicate a significant difference ($p = 0.05$).
^b *, **, ***, and ns indicate significance at $p \leq 0.05$, ≤ 0.01 , ≤ 0.001 , and not significant, respectively.

was observed for 11 amino acids (not including proline) to N timing and was particularly pronounced for alanine (ALA) and arginine (ARG), where the concentrations were doubled from the first (N1) to the second last timing of N application (N5) (Tab. 4). In contrast, GABA was highest when N was applied after full bloom (N1) and lowest when N was applied two weeks prior to harvest (N5). N timing had no effect on a number of amino acids, namely PHE, histidine (HIS), leucine (LEU), iso-leucine (ILE) and MET. There were significant interactions between water and N application, for the amino acids serine (SER) and glycine (GLY), with the lowest concentrations found under full irrigation in combination with the after full bloom N application (N1). Conversely, the highest levels were apparent under full water supply and the N application four weeks prior to harvest (N5). The influence of N supply on berry amino N has been described earlier (HOLZAPFEL and TREEBY 2007), where it was shown that amount and timing can impact on juice amino acid concentrations. In contrast to this study, limited water supply can reduce juice amino acid N (WADE *et al.* 2004), and this could be due to the reduced allocation of N to the annual vegetative parts. The alterations in the amino acids concentrations though these practical inputs suggests an effect on amino acid metabolism within the berry. These findings demonstrate that N application and water supply can alter amino acid concentrations and its metabolism. Overall, the timing of N application can be critical to YAN levels at harvest, with consequences on fermentation and final wine composition.

Conclusions

The timing of N application during berry development altered vine growth, berry size development and concentrations of berry nitrogenous compounds at harvest. N application shortly after flowering led to a substantial increase in shoot growth and yield. The later was due to an enhanced fruit-set, despite the elevated shoot growth in this period competing for assimilates. The partitioning of N between the annual and perennial portions of the vines was influenced by both water supply and time of N application. The annual components of the vine, as determined at harvest, contained more N after full irrigation (FI), but also with the earliest N application which occurred at 70 d after budburst (N1). In contrast, under limited irrigation (HI) and later N application the perennial structure accumulated more N. In addition, the vines that received half the irrigation produced berries that had elevated NH₄ levels in the juice, while the timing of the N application had no influence. The best time to administer N in relation to YAN concentration of the juice, was four weeks prior to harvest in this study. This suggests that fruit N could be elevated later in the season through soil N application if required, without the adverse effects of accelerated shoot growth. The findings suggest that water availability and timing of N supply can alter the concentrations of berry amino N. In addition, these two management tools can influence the N root reserves which are likely to impact on the development of the vine and the fruit in the following season.

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