Timing of Nitrogen Fertilisation and the Effect of Poultry Manure on the Performance of Grapevines on Sandy Soil. II. Leaf Analysis, Juice Analysis and Wine Quality*

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The effects of timing of nitrogen fertilisation and of different sources (organic *vs* inorganic) on leaf analysis, juice analysis and wine quality for grapevines on a sandy soil with low organic material were investigated over a period of nine years. Two *Vitis vinifera* L. cultivars, *viz.* Bukettraube (white) and Heroldrebe (red), both grafted onto Ramsey, were used. The control (N_0) received no N, while three treatments each received 50 kg N/ha in inorganic form. The N was applied either as three equal installments, split between budbreak, fruitset and post-harvest (N_1), or as a single application at budbreak (N_2), or as a single post-harvest application (N_3). A fifth treatment (N_4) received 50 kg N/ha at budbreak in the form of poultry manure. Total N in leaf blades and petioles, sampled at fruitset, tended to be highest for N_2 and lowest for N_0, but differences were relatively small. The NO_3-N in petioles showed larger differences, with the value for N_0 generally being significantly lower than that of inorganically fertilised treatments. A single application of N at budbreak (N_2), delayed maturity for both cultivars, while this was also the case for split applications (N_1) for the more vigorous cultivar (Bukettraube). Total N in juice was lowest for N_0 and N_4 and higher for N_1, N_2 and N_3, for both cultivars. In the case of Bukettraube, assimilable N was lowest for N_0, while the other treatments did not differ. For Heroldrebe, with a lower sugar content, assimilable N was lowest for N_0, N_2 and N_4 and higher for N_1 and N_3. Arginine was the predominant amino acid, constituting 61% and 40% of total amino-N for Bukettraube and Heroldrebe, respectively. Wine quality (Bukettraube only) was always lowest for N_0 and higher for N_1, N_2 and N_3. During most seasons quality did not differ between the latter three treatments. However, with N_2 being more prone to fungal diseases, wine quality was reduced for this treatment when climatic conditions favoured *Botrytis cinerea* infection. Wine from the organic treatment (N_4) scored marginally higher than N_1, N_2 and N_3. Under the conditions of this trial, split applications of N (budbreak, fruitset, post-harvest) or a single application during the post-harvest period, or application of organic N ensured highest wine quality. Reaction to N may be different for less vigorous rootstocks.

Nitrogen (N) is required for yeast growth and fermentation, and is one of the most important nutrients in must. If juice contains too little available N, stuck fermentation can occur. In some cases this will lead to the formation of undesirable components, such as H_2S (Vos & Gray, 1979). The minimum N requirement for must, *i.e.* the concentration of assimilable N below which the rate of fermentation is unsatisfactory, has been reported to be 120-140 mg/L (Agenbach, 1977; Spayd, Nagel & Edwards, 1995). Fermentation initiated with twice this concentration of N may nonetheless also produce H_2S, and the amount of assimilable N utilised when all amino acids are in excess has been estimated at 400 mg/L (Jiranek, Langridge & Henschke, 1995). On the other hand, high juice-N need not necessarily be beneficial to wine quality. A high content of residual N in must may encourage microbial instability (Jiranek *et al.*, 1995) and ethyl carbamate accumulation in wine (Ough, 1991; Henschke & Jiranek, 1993). The ideal N fertilisation programme should ensure an optimal level of assimilable N in juice, sufficient to eliminate the above-mentioned negative characteristics.

The N content of must can also have a direct, or an indirect, effect on aroma or "wine bouquet". The main groups of compounds that form the "fermentation bouquet" are the organic acids, higher alcohols and esters, which are influenced to various degrees by the nitrogen source (Rapp & Versini, 1991). The higher alcohols generally have a negative effect on wine quality (Henschke & Jiranek, 1993). This negative effect is reduced at high juice-N levels (Ough & Bell, 1980; Webster *et al.*, 1993). The majority of esters normally have a positive effect on wine quality (Henschke & Jiranek, 1993). This positive effect increases with increasing juice-N levels (Ough & Lee, 1981; Webster *et al.*, 1993). Furthermore, as reviewed by Henschke & Jiranek (1993), nitrogen compounds, such as the products of cell autolysis, may themselves contribute to, or modify, wine flavour. Even though the aroma of wine is influenced by several hundred different components (Rapp & Versini, 1991), the above-mentioned factors may have contributed to the fact that sensory evaluations pointed towards higher wine quality in cases where the total juice-N was increased through N fertilisation (Bell, Ough & Kliewer, 1979; Goldspink & Frayne, 1993; Larchevêque *et al.*, 1998).

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Because of the relationship between the assimilable N content of must and the final sensory quality of wine, interest has lately turned towards the nitrogen composition of grapes. Amino acids account for between 60% and 90% of total juice-N (Kliewer, 1969), and it is known that individual amino acids may influence the flavour composition of wine (Rapp & Versini, 1991). Consequently, the amino acid profiles of *Vitis* species have been evaluated in many regions and were found to vary with cultivation practices and growing region (Hernández-Orte, Guitart & Cacho, 1999; and references therein). In some cases arginine was found to be the predominant amino acid, while proline was the major amino acid in others (Huang & Ough, 1991; Spayd & Andersen-Bagge, 1996; Stines *et al.*, 2000). With proline normally being non-assimilable under enological conditions, it is of special importance to know its contribution to total amino-N. The term "assimilable N", as used in the rest of this paper, will refer to total amino-N and ammonia-N, minus proline-N.

Conflicting results regarding the effects of N fertilisation on the nitrogenous compounds in grapes have been reported, probably because cultivar, soil-N content, cultural practices and climatic factors may override the effectiveness of N fertiliser (Spayd *et al.*, 1994). In South Africa, on soil with a medium organic content, application of 56 kg N/ha, as compared to 16 kg N/ha, increased total N in juice by only 5% (Conradie & Saayman, 1989). In Washington, on soil with a low organic material content, total juice-N increased linearly with increasing N fertiliser rates up to 224 kg N/ha (Spayd *et al.*, 1994). It has also been widely reported that excessive N fertilisation may enhance susceptibility to *Botrytis cinerea* (Conradie & Saayman, 1989; Delas, Molot & Soyer, 1991; Christensen *et al.*, 1994; Spayd *et al.*, 1994). Such infection influences the amino acid content of berries and/or juice, and total amino acid content can decrease by up to 80% (Rapp & Versini, 1991; and references therein).

In a South African investigation on sandy soil with low organic material application of 50 kg N/ha increased grape yield for Bukettraube (white) and Heroldrebe (red) by 24% and 48%, respectively (Conradie, 2001). Time of application (budbreak, fruitset, post-harvest) or source of N (organic *vs* inorganic) did not affect yield response, but a single application at budbreak did cause excessive shoot growth and enhanced the occurrence of botrytis during some seasons. In conjunction with the above-mentioned investigation, the first objective of this study was to determine the effect of different N fertilisation practices on juice composition (including amino acid profiles), and on wine quality, for sandy soil with a low organic material content. The study was carried out in accordance with typical South African cultivation practices.

Secondly, the existence of a possible relationship between leaf analysis (carried out during the active growing season) and assimilable N, as determined in juice at harvest, was to be investigated. If such a relationship does exist, time will be available to correct N deficiencies before harvest. In Washington, NO_3-N in petioles at bloom correlated with the N concentration in Riesling juice (Spayd *et al.*, 1995), but in Australia this was not the case for Chardonnay (Treeby *et al.*, 1998). In South Africa the relationship between NO_3-N in petioles and arginine in fruit has been studied for Thompson Seedless on various rootstocks (Kliewer, 1991), but little is known about the relationship in wine grapes.

MATERIALS AND METHODS

Experimental layout

Experimental layout and experimental procedures were previously described (Conradie, 2001). Briefly, the trial was carried out in the Stellenbosch district on a sandy soil (7% clay) with low organic material (0.27% C). Two *Vitis vinifera* L. cultivars, Bukettraube (white) and Heroldrebe (red), both grafted onto Ramsey, were planted in a split-plot design. Five fertilisation treatments (Table 1), each replicated three times, were applied from 1981/82 to 1989/90. Fertilisers were applied by hand at the relevant phenological stages. The poultry manure (PM) contained 1.7% K, which was compensated for in the other treatments by the application of potassium chloride (Conradie, 2001). Rye was sown annually as a winter cover crop, killed with glyphosate prior to budbreak and flattened to form an organic mulch on the soil surface. During summer the vines received two overhead sprinkler irrigations of about 60 mm each. In order to schedule these irrigations soil water content was determined gravimetrically, once every two weeks, during November, December and January. The first irrigation was normally required at fruitset (defined as two weeks after the end of bloom) and the second by middle January (approximately one month before harvest).

TABLE 1

Source, quantity and timing of fertiliser nitrogen applied from 1981/82 to 1989/90.

Treatment	Source of Nitrogen	Nitrogen applied (kg N/ha)		
		Budbreak (1)	Fruitset	Post-harvest
N_0	–	0	0	0
N_1	Inorganic (2)	17	16	17
N_2	Inorganic	50	0	0
N_3	Inorganic	0	0	50
N_4	Organic (3)	50	0	0

(1) Applied three weeks after actual date of budbreak.
(2) Applied as limestone ammonium nitrate (28% N).
(3) Applied as poultry manure.

Leaf analysis

Leaves (petioles and blades), directly opposite clusters, were sampled at fruitset. Total N was determined in a selenous acid/sulphuric acid digest by means of an automated colorimetric method (The Non-Affiliated Soil Analysis Work Committee, 1990). Plant tissue-P, -K, -Ca and -Mg were determined following dry ashing in a microwave furnace and uptake into acidified aqueous solution using a Varian 200 inductively coupled plasma atomic emission spectrometer. Petiole samples from 1989/90 were also extracted with 1.0 M KCl and analysed for NO_3N, using a colorimetric method (The Non-Affiliated Soil Analysis Work Committee, 1990).

Juice analysis

From 1983/84 to 1989/90 grapes were harvested when the sugar concentration of Bukettraube averaged 21°B. All treatments were harvested on the same date. A representative sample (at least ten

bunches) from each plot was crushed in a hydraulic press. Free-run juice was analysed for sugar content (temperature compensated Abbé refractometer), pH (654 Metrohm pH meter) and titratable acidity (50 mL juice titrated with 0.333 M NaOH to pH 7.0 and expressed as g tartaric acid/L). Total juice-N was determined by means of an automated colorimetric method (The Non-Affiliated Soil Analysis Work Committee, 1990), following digestion with selenous acid/sulphuric acid. Total P, K, Ca and Mg levels in juice were determined by means of atomic absorption spectrophotometry, following digestion with nitric acid/perchloric acid. During 1987/88 and 1988/89 free amino acids were also determined. Acid, neutral and basic amino acids were separated on two different columns packed with a cation exchange phase, as described previously by Moore, Spackman & Stein (1958), using an amino acid auto-analyer. Operating conditions have been described previously (Kluba, Mattick & Hackler, 1978).

Experimental wines

Due to the low sugar content and low yield of Heroldrebe, experimental wines were prepared for Bukettraube only for four years (1986/87 to 1989/90). Forty to 60 kg of grapes were harvested from each plot. During the first two seasons (1986/87 and 1987/88) grapes were crushed, de-stemmed and immediately pressed to 1 bar in a small-scale pneumatic press. Sulfur dioxide was adjusted to 50 mg/L and 0.5 g/hL Ultrazyme was added, before settling overnight at 14°C. Clear juice was drawn off into 20 L stainless steel canisters, and inoculated with a strain of *Saccharomyces cerevisiae* (VIN 13), at 30 g/hL. Fermentation took place at 14°C and bentonite (75 g/hL) was added two days after commencement of fermentation. Wines were fermented to dryness, as tested with a Clinistix™ strip (Bayer, Cape Town), at which point 50 mg/L of SO_2 was added. Wines were racked two days later and free SO_2 was adjusted to 35 mg/L. Wines were cold stabilised at 0°C for a minimum of one week, after which they were racked, and filtered through K700 and EK filter sheets before being bottled in 750 mL bottles. Free SO_2 was adjusted to 40 mg/L at bottling and the wines were stored at 14°C for three months before being evaluated. During 1988/89 and 1989/90 the grapes underwent a skin contact period of six hours at 14°C, after being crushed and de-stemmed. The rest of the procedure was as described above. Sensory evaluation of wines was carried out by an experienced panel of 14 members on a nine-point score card (Tromp & Conradie, 1979). Wines, presented in coded form, were scored for overall wine quality and cultivar character. In the latter case a reference sample, regarded as being typical of Bukettraube, was also supplied.

Statistical analysis

Data was analysed statistically and Student's t LSD values were calculated at the 5% level of probability to facilitate comparison between treatment means.

RESULTS AND DISCUSSION

Leaf analysis

The nutrient content of leaves fluctuated from season to season, but trends remained fairly consistent and the mean values for 1983/84 to 1989/90 are shown in Table 2. Total N in leaf blades from Bukettraube tended to be higher where N was applied during spring (N_1 and N_2), but differences were not significant. For Heroldrebe, however, leaf blades from N_2 (single application at

TABLE 2

Effect of application time and source of N on the nutrient content of leaf blades and petioles for Bukettraube and Heroldrebe (Means for 1983/84 to 1989/90).

Treatment [1]	Organ	Bukettraube						Heroldrebe					
		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	NO_3-N[3] (mg/kg)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	NO_3-N (mg/kg)
N_0 – Control	Blade	2.46 a[2]	0.27 a	0.92 a	1.46 ab	0.31 ab	–	2.15 a	0.24 a	0.68 ab	1.35 ab	0.33 ab	–
N_1 – Split application	Blade	2.71 a	0.25 a	0.96 a	1.46 ab	0.34 a	–	2.26 ab	0.20 a	0.63 a	1.32 a	0.35 a	–
N_2 – Single application at budbreak	Blade	2.72 a	0.24 a	0.94 a	1.67 c	0.30 ab	–	2.36 b	0.21 a	0.67 a	1.62 c	0.32 ab	–
N_3 – Single post-harvest application	Blade	2.56 a	0.26 a	0.88 a	1.64 bc	0.29 b	–	2.25 ab	0.22 a	0.65 a	1.52 bc	0.30 b	–
N_4 – Poultry manure as N-source	Blade	2.53 a	0.27 a	1.10 b	1.39 a	0.33 ab	–	2.29 ab	0.24 a	0.84 b	1.30 a	0.31 ab	–
N_0 – Control	Petiole	0.63 a	0.32 a	1.24 a	1.22 a	0.68 ab	8.3 a	0.63 a	0.24 a	0.88 a	0.98 a	0.48 a	22.7 a
N_1 – Split application	Petiole	0.65 a	0.28 a	1.16 a	1.17 a	0.71 b	28.2 ab	0.64 a	0.18 b	0.72 b	0.98 a	0.51 a	68.6 b
N_2 – Single application at budbreak	Petiole	0.66 a	0.27 a	1.21 a	1.39 b	0.60 b	53.9 b	0.67 a	0.21 ab	0.95 a	1.19 b	0.46 a	60.8 b
N_3 – Single post-harvest application	Petiole	0.66 a	0.29 a	1.22 a	1.41 b	0.58 a	37.7 ab	0.63 a	0.22 ab	0.91 a	1.15 bc	0.46 a	42.3 ab
N_4 – Poultry manure as N-source	Petiole	0.67 a	0.31 a	1.42 b	1.07 a	0.68 ab	27.2 ab	0.65 a	0.22 ab	1.18 c	0.99 ac	0.44 a	34.8 ab

(1) See Table 1 for full details of treatments.
(2) Values within columns, within plant organs, followed by the same letter do not differ significantly ($\rho \leq 0.05$)
(3) Determined in 1989/90 only.

budbreak) contained more N than those from the control (N_0). The same pattern applied, albeit to a lesser extent, for total N in petioles. Local norms of 1.6% and 0.6%, regarded as the deficiency levels for blades and petioles, respectively (Conradie, 1986a), were exceeded for all treatments. In view of the fact that the control yielded significantly less than the fertilised treatments (Conradie, 2001), mainly on account of N deficiency, total N in blades and petioles appeared to be a poor guideline for determination of N nutritional status. Values for unfertilised Chenin blanc on soil with a higher organic content (0.65% C) actually tended to be lower (Conradie & Saayman, 1989) than those obtained in this study for soil with a low organic content (Table 2). This confirmed that different norms should be used under different situations and for different scion/rootstock combinations (Conradie, 1986a).

The NO_3-N in petioles (Table 2), was low in comparison to values obtained at bloom for table grapes under intensive irrigation in Hex River Valley (Conradie & Van Huyssteen, 1996). However, in the present study leaves were sampled before the first irrigation, at which point the soil was relatively dry (Conradie, 2001), thereby restricting the uptake of N. Under such conditions a low value for NO_3-N in petioles does not necessarily indicate soil-N deficiency (W.J. Conradie, ARC-Nietvoorbij, unpublished data). Relatively low NO_3-N values have also been obtained for wine grape cultivars in other countries (Goldspink & Gordon, 1991; Christensen *et al.*, 1994; Spayd *et al.*, 1993). Despite the low NO_3-N values results still confirmed that the difference in total N between control vines and N-fertilised vines can be in the order of 10% only, whereas the range for NO_3-N can be 100% or more (Kliewer, 1991). In the case of Bukettraube the value for N_2 was significantly higher than that of the control, while both N_1 and N_2 showed higher NO_3-N for Heroldrebe. In this trial "budbreak" N was applied 3-4 weeks after the actual date of budbreak and there was clearly sufficient time for N to be washed into the soil by rainfall and to be absorbed by fruitset. Similar results were obtained elsewhere (Goldspink & Gordon, 1991; Christensen *et al.*, 1994). Where N was applied during the post-harvest period (N_3), NO_3-N in petioles did not differ from that of N_2, even though values tended to be lower. It has been shown that roots and other permanent parts serve as important storage organs for N during the post-harvest period (Conradie, 1980; Löhnertz, 1988; Peacock, Christensen & Broadbent, 1989; Conradie, 1991; Williams, 1991). These reserves are utilised by new growth in spring, even when adequate N is available in the soil. Under the conditions of this trial, post-harvest fertilisation thus proved to be just as effective as split applications or a single application at budbreak. This is in agreement with previous results (Conradie, 1986b; Peacock, Christensen & Hirschfelt, 1991; Christensen *et al.*, 1994). Where post-harvest fertilisation proved less effective (Goldspink & Gordon, 1991; Treeby, Holzapfel & Walker, 1995), it can possibly be ascribed to differences in climate, cultivation practices and scion/rootstock combinations. Soil water status, especially, can have a major effect on the uptake of N (Gockowiak & Henschke, 1992). In the current trial adequate rain during autumn probably enhanced uptake of N applied during the post-harvest period, while insufficient soil water during some stages of the pre-harvest period (Conradie, 2001) may have had a negative effect on the uptake of N applied at fruitset. The NO_3-N in petioles from the poultry manure treatment (N_4) tended to be lower, albeit not significantly, than that of the other fertilised treatments. This suggested that the poultry manure (50 kg N/ha in organic form) supplied less inorganic N than the inorganically fertilised treatments.

Split N applications (N_1) reduced the P-content in Heroldrebe petioles, as was found previously (Conradie & Saayman, 1989; Spayd *et al.*, 1993). However, P seemed to be adequately supplied in all cases (Conradie, 1986a). Application of poultry manure (N_4), which increased soil-K (Conradie, 2001), led to higher K concentrations in blades and petioles (Table 2). Even though soil-K did not appear to be critically low for the inorganic treatments (Conradie, 2001), maintenance K fertilisation (27 kg K/ha) was apparently inadequate. In view of the locally accepted deficiency levels of 0.65% for blades and 1.00% for petioles (Conradie, 1986a), the petioles from Heroldrebe indicated a K deficiency. The red cultivar was more susceptible to K deficiency than the white one. Calcium concentrations for both cultivars were higher where N was applied as a single increment, either at budbreak or post-harvest. The reason for this is unclear, but a marginal increase in Ca concentration, due to N fertilisation, was reported previously (Conradie & Saayman, 1989). Applications of N during the post-harvest period appeared to depress Mg concentrations, but the effect was minor and unlikely to be of practical importance.

Juice analysis

The sugar content of Heroldrebe, a relatively late ripening cultivar, was approximately 3°B lower than that of Bukettraube (Table 3). For both cultivars the lowest value was obtained where N was

TABLE 3

Effect of application time and source of N on sugar, titratable acidity and pH of juice, for Bukettraube and Heroldrebe (Means for 1982/83 to 1989/90).

Treatment [1]	Bukettraube			Heroldrebe		
	Sugar (°B)	Total Acids (g/L)	pH	Sugar (°B)	Total Acids (g/L)	pH
N_0 – Control	22.3 a [2]	9.26 a	3.07 a	18.5 ab	6.90 a	3.22 a
N_1 – Split application	20.9 bc	9.45 a	3.05 a	18.7 a	6.80 a	3.25 a
N_2 – Single application at budbreak	20.0 c	10.49 b	3.06 a	17.7 b	7.71 b	3.21 a
N_3 – Single post-harvest application	21.3 ab	9.61 a	3.06 a	18.5 ab	6.99 a	3.24 a
N_4 – Poultry manure as N-source	21.3 ab	9.51 a	3.07 a	18.5 ab	7.59 b	3.24 a

[1] See Table 1 for full details of treatments.

[2] Values within columns followed by the same letter do not differ significantly ($p \leq 0.05$).

applied in a single application at budbreak. For the more vigorous cultivar (Bukettraube) split applications (N_1) also reduced the sugar content. As grape yield was comparable for all the fertilised treatments (Conradie, 2001), lower sugar contents could not be ascribed to differences in crop size. However, delayed maturation, due to N fertilisation, was also reported by others (Peacock *et al.*, 1991; Christensen *et al.*, 1994; Spayd *et al.*, 1994). This agrees with the theory of Champagnol (1978) that increased vegetative growth, as measured for N_1 and N_2 (Conradie, 2001), can delay maturity. Sugar concentration was not affected where N was supplied either as a single inorganic application during the post-harvest period, or as a single organic application at budbreak. Total acidity was higher for N_2, thus agreeing with the lower sugar content. The higher acid content obtained for N_4 in the case of Heroldrebe may have been due to the higher K content of the soil (Conradie, 2001). None of the treatments affected the pH of the juice.

Juice from Bukettraube contained more total N than that from Heroldrebe (Table 4). This may have been on account of different sugar contents (Hernández-Orte *et al.*, 1999) or due to differences in N partitioning between the two cultivars (Stines *et al.*, 2000). For both cultivars a single post-harvest application of N was as effective as a single application at budbreak in increasing juice-N. This is in contrast to results obtained in Australia (Goldspink & Gordon, 1991) for Sauvignon blanc grafted onto Schwarzmann rootstock, where post-harvest fertilisation was less effective. It is known, however, that different responses can be obtained for different scion/rootstock combinations. Where Ramsey (Treeby *et al.*, 1995) or 420A (Berger *et al.*, 1999) was used as rootstock, N fertilisation appeared to have less effect on juice-N. In the current trial, where Ramsey was used, fertilisation increased juice-N by less than 20% for the more vigorous cultivar (Bukettraube), while an increase of nearly 30% was found for the less vigorous one (Heroldrebe). Furthermore, for own-rooted White Riesling on soil with 0.3% organic material (thus being comparable to the current soil), juice-N more than doubled when N fertilisation was increased from zero to 56 kg/ha (Spayd *et al.*, 1994). It has also been shown that the effect of N fertilisation on nitrogenous compounds in juice may be largely influenced by soil water status and cultivation practices (Gockowiak & Henschke, 1992; Larchevêque *et al.*, 1998). Above-mentioned results suggested that vine vigour, being largely affected by scion/rootstock combinations as well as by soil-N content, cultural practices and climatic factors, can override the effectiveness of N fertiliser to increase juice-N.

Split applications of N (N_1) had a minor (insignificant) effect on juice-N, especially in the case of Bukettraube. This is in agreement with the low value for NO_3-N in petioles (Table 2), suggesting that fertilisation at fruitset was not effective on this sandy soil. Two irrigations, as applied in this study, were probably insufficient and incapable of maximising the uptake of N applied at fruitset. Soil water content decreased to wilting point during the period preceding the second irrigation, as well as the last 2-3 weeks before harvest (Conradie, 2001). A better response to fertilisation at fruitset can be expected under more intensive irrigation, provided that no N is leached from the root zone.

In contrast to the positive response observed for N_2 and N_3, organic fertilisation (N_4) did not increase juice-N, in comparison to N_0. One of the main differences between N_0 and N_4 was an adequate supply of N from budbreak to bloom in the case of the organic treatment (Conradie, 2001). Consequently, yield of the N_4 treatment was significantly higher than that of N_0. However, during the latter part of the season (bloom to harvest) the N_4 treatment must have supplied less N to bunches in comparison to N_2 and N_3.

In general, juice-N correlated with NO_3-N in petioles, as measured at fruitset (Table 2). This was in agreement with results obtained by Spayd *et al.* (1995). Treatments with the lowest NO_3-N resulted in lowest juice-N. The "threshold value" for NO_3-N appeared to have been around 35 mg/kg. If this value had been used as a yardstick, N_0 and N_4 (both cultivars) and N_1 (Bukettraube only) would have been fertilised at fruitset. However, fertilisation at fruitset should be accompanied by adequate irrigation practices.

Nitrogen fertilisation did not affect the P content of the juice, which is in accordance with previous results (Conradie & Saayman, 1989). Despite leaf analysis (Table 2) indicating a higher K concentration for N_4, no significant differences could be detected for juice-K. Apparently the K status of the inorganic treatments (both soil and leaves) was still high enough to supply sufficient K to the bunches from fruitset to harvest. The two treatments with highest leaf-Ca (N_2 & N_3) did not cause an increase in juice-Ca. However, juice-Mg was lower for both these treatments, pointing towards Ca/Mg antagonism. Both Ca and Mg concentrations were of the same order as those found previously in must (Conradie & Saayman, 1989; Larchevêque *et al.*, 1998).

In general, total juice-N concentrations for 1987/88 and 1988/89 (Table 5) were in good agreement with average values obtained

TABLE 4

Effect of application time and source of N on juice composition for Bukettraube and Heroldrebe (Means for 1982/93 to 1989/90).

Treatment [1]	Bukettraube					Heroldrebe				
	N (mg/L)	P(mg/L)	K(mg/L)	Ca(mg/L)	Mg(mg/L)	N(mg/L)	P(mg/L)	K(mg/L)	Ca(mg/L)	Mg(mg/L)
N_0 – Control	505 a[2]	148 a	1533 a	42 a	70 bc	381 a	143 a	1664 a	39 a	59 a
N_1 – Split application	528 ab	128 a	1505 a	40 a	72c	442 bc	130 a	1530 a	32 a	55 ab
N_2 – Single application at budbreak	572 b	135 a	1489 a	42 a	65 ab	478 c	137 a	158 5a	37 a	51 b
N_3 – Single post-harvest application	572 b	142 a	1467 a	44 a	62 a	488 c	139 a	1574 a	37 a	52 b
N_4 – Poultry manure as N-source	497 a	138 a	1549 a	39 a	72 c	401 ab	131 a	1593 a	36 a	58 a

(1) See Table 1 for full details of treatments.
(2) Values within columns followed by the same letter do not differ significantly ($p \leq 0.05$).

over the whole investigation period (Table 4), while amino acid profiles were fairly consistent for the two seasons. The fact that grapes from N_2 were infected with botrytis during 1988/89 (Conradie, 2001) did not appear to affect the distribution of amino acids (data not shown). Consequently, only mean values are shown in Table 5. In the case of Bukettraube, amino-N concentrations in juices from the control and the inorganically fertilised treatments, showed similar tendencies to those exhibited by total juice-N. Juice from the organically fertilised treatment, however, also showed high amino-N, in spite of the low total N. Virtually all N in juice from this treatment (96.5%) as well as the control treatment (96.7%) appeared to be present in the form of either amino-N or ammonia-N. It has been reported that amino-N can constitute 50-90% of the total N present in must (Kliewer, 1969). The reason why the ratio between assimilable N and non-assimilable N varied to such a large extent between treatments was unclear. However, little is known about the factors affecting the distribution between assimilable N (mainly amino acids) and non-assimilable N (mainly peptides and proteins). The protein-N in juice from White Riesling in Washington increased significantly when inorganic fertilisers were applied (Spayd *et al.*, 1994). In the Washington study as well as the present one (Conradie, 2001) inorganic N fertilisation caused increased shoot growth. A study in Germany showed that juice contained a higher proportion of assimilable N (amino acids) during cooler years, due to less proteins being synthesised (Schrader *et al.*, 1976). In Germany less vigorous growth can normally be expected during cooler years. Less vigour and higher ratios for yield to shoot mass (Y:SM) in the cases of N_0 and N_4 (Conradie, 2001) may have induced different ratios between assimilable and non-assimilable N. This is also in agreement with the suggestion that free amino-N in must from South African vineyards tends to be higher where organic fertiliser is applied (P.J.A. Vos, personal communication, 1999). Results suggested that improved uptake of N, as implied by NO_3-N in petioles of inorganically fertilised treatments (Table 2), may increase total juice-N, but not necessarily the concentration of assimilable N. Even though assimilable N was lowest for the control, all values for Bukettraube exceeded 400 mg/L, indicating that fermentation should not be impeded (Jiranek *et al.*, 1995).

For Heroldrebe, in contrast to Bukettraube, amino-N was lower for N_2 than for N_1 and N_3. Again, this may have been due to the sugar content of this treatment (N_2) being about 1°B lower than that of the other two. Due to arginine being the predominant amino acid (Table 5), amino-N may well have increased, if the grapes had been allowed to attain full maturity (Hernández-Orte *et al.*, 1999; Stines *et al.*, 2000). For this cultivar amino-N accounted for a smaller fraction of total juice-N and assimilable N was less than 400 mg/L for all treatments. In contrast to Bukettraube, organic fertilisation did not appear to have a beneficial effect on the distribution between assimilable and non-assimilable N for this less vigorous cultivar.

TABLE 5

Effect of application time and source of N on amino acids and total N in juice from Bukettraube and Heroldrebe (Means for 1987/88 and 1988/89).

Compound (mg N/L)	Bukettraube					Heroldrebe				
	N_0 (1)	N_1	N_2	N_3	N_4	N_0	N_1	N_2	N_3	N_4
Alanine	13.9	15.2	17.1	14.9	16.6	17.5	22.9	19.0	25.8	20.1
γ-Amino butryric acid	27.2	29.3	29.4	29.1	32.9	23.3	23.7	31.0	34.7	25.8
Arginine	270.6	310.7	318.9	296.5	304.6	108.1	178.9	133.5	180.7	115.4
Asparagine	1.1	1.3	2.8	1.7	1.8	4.7	4.6	4.4	5.7	4.5
Aspartic acid	2.7	2.3	3.6	2.3	2.7	3.2	3.6	2.5	3.4	3.8
Glutamic acid	2.6	2.6	3.9	2.7	2.9	3.6	3.6	2.7	3.4	3.5
Glutamine	14.2	17.0	20.7	16.8	17.0	22.0	14.6	19.7	26.3	23.6
Histidine	13.5	15.2	15.3	13.7	14.4	7.5	11.4	8.8	11.2	7.9
Isoleucine	1.8	2.2	2.1	1.8	2.0	1.5	2.1	1.8	2.2	1.7
Leucine	3.4	3.5	3.4	3.1	3.6	2.5	3.2	2.9	3.4	2.6
Ornithine	2.9	3.3	3.8	2.8	3.3	1.0	1.6	1.2	1.4	1.3
Phenylalanine	2.1	1.9	2.8	2.0	1.9	0.7	0.8	0.8	0.9	0.7
Proline	30.0	29.5	24.8	24.5	29.5	53.2	58.0	49.4	66.1	61.1
Serine	5.4	6.2	7.3	5.5	6.7	6.0	7.4	6.5	8.4	6.8
Threonine	7.9	10.1	10.0	8.4	9.8	5.6	8.8	7.1	8.9	6.5
Valine	4.0	4.3	4.4	3.7	4.0	2.7	3.6	3.3	4.1	3.1
Others (2)	9.2	10.0	9.8	10.1	8.2	9.3	10.3	13.0	14.8	8.6
Ammonia	30.9	37.8	38.9	41.8	37.1	34.2	33.9	30.4	43.2	35.2
Total amino-N (3)	**443**	**502**	**519**	**481**	**499**	**307**	**393**	**338**	**445**	**332**
Assimilable N (4)	**413**	**473**	**494**	**456**	**469**	**254**	**335**	**289**	**379**	**271**
Total N	**458**	**553**	**595**	**588**	**517**	**401**	**508**	**501**	**493**	**411**

(1) See Table 1 for full details of treatments.

(2) Glycine, cysteine, methionine, beta alanine, lysine, tryptophane and tyrosine.

(3) Including ammonia-N

(4) Excluding proline-N

In all Bukettraube treatments arginine accounted for approximately 61% of total amino acids and proline for 5% to 6%. These two are usually the major amino acids in grapes (Huang & Ough, 1991; Hernández-Orte *et al.*, 1999; Stines *et al.*, 2000), while a value of 65% for arginine + proline has also been reported by others (Larchevêque *et al.*, 1998). The next most abundant amino acids for Bukettraube were: γ-amino butyric acid, glutamine, alanine and histidine. The contribution of these four, plus arginine and proline, to the total amino-N pool was remarkably consistent, amounting 82% to 83% for all five treatments. In general, these six amino acids have also been identified by others (Henschke & Jiranek, 1993; Larchevêque *et al.*, 1998; Hernández-Orte *et al.*, 1999; Stines *et al.*, 2000) as frequently dominating in grape juice. The most notable difference between the results in Table 5 and those of above-mentioned authors is that they often found appreciable amounts of glutamic acid as well. However, it has been shown that the concentration of glutamic acid can vary appreciably from season to season (Larchevêque *et al.*, 1998), possibly on account of changes in climatic conditions. Higher amino-N for fertilised treatments (Table 5) was largely due to increased concentrations of arginine, while proline was not affected. This is in agreement with the results obtained for Shiraz/Ramsey (Treeby *et al.*, 1995), where N fertilisation increased assimilable N, while this was not necessarily the case for non-assimilable N.

For Heroldrebe arginine contributed 35% to 45% towards amino-N and proline 15% to 18%, indicating that the arginine: proline ratio was considerably lower for this cultivar than for Bukettraube. The four next most abundant amino acids for Heroldrebe were the same as for Bukettraube, even though the concentration of histidine appeared to be less. The contribution of the six most abundant amino acids (arginine, proline, γ-amino butyric acid, glutamine, alanine and histidine) varied from 75.4% (N_0) to 78.8% (N_1), thus being marginally lower than the value found for Bukettraube. As in the case of Bukettraube, higher amino-N for treatments N_1 and N_3 (Table 5) could largely be ascribed to increased concentrations of arginine.

It has been suggested that the basic pattern of amino acid composition in mature fruit is determined by genetic factors and that environmental and cultural factors have only a modifying effect (Stines *et al.*, 2000). No previous work on the amino acid profiles of Bukettraube and Heroldrebe has been done. However, arginine appears to be dominant in Bukettraube, while the concentration of proline is much lower. Moderate levels of both arginine and proline can be expected for Heroldrebe.

Wine quality

During 1986/87 and 1987/88, when wines were made without any skin contact, quality was poor (data not shown) and no differences could be detected. In 1988/89, when wines were prepared with skin contact, N_0 and N_2 were inferior to N_3 and N_4 (Table 6). The panel regarded N_0 as "flat", without any prominent cultivar characteristics. In the case of N_2 wine was regarded as having an "off" taste, probably on account of grapes from this treatment being infected by *Botrytis cinerea* during this season. Overall wine quality did not differ in 1989/90, but N_0 again received the lowest score for cultivar character. Botrytis was not a problem during 1989/90 and the inorganically fertilised treatments (N_1, N_2 and N_3) showed no significant differences. Even though N_4 did not differ significantly from N_1, N_2 and N_3, overall quality (1988/89 and 1989/90), as well as cultivar character (1989/90), tended to be the highest. In ranking tests (data not shown) the majority of the panel members regarded wine from N_4 as being slightly superior to wines from inorganically fertilised treatments. The reason for this was unclear, in view of the fact that total juice-N was relatively low for N_4 (Table 3), while assimilable N was, at best, comparable to that of N_1, N_2 and N_3. Higher wine quality may have been due to the formation of additional aromatic substances. Analytical determination of such substances was outside the scope of this study. These results, however, did illustrate the difficulty of being able to define "wine quality" solely using analytical data (Webster *et al.*, 1993). Relating analytical data to wine quality is complicated by the fact that interactions occur between aroma compounds, which affect sensory evaluation.

Strategy for N fertilisation on sandy soils

Results from this, and from the previous study (Conradie, 2001), showed that a single application of N, either at budbreak or post-harvest, or incremental applications are equally effective in increasing yield for grapevines (grafted onto Ramsey) on sandy soil, while juice also contained comparable amounts of assimilable N. However, leaf analysis done at fruitset showed higher N uptake, where N was applied as a single increment at budbreak, resulting in more vigorous shoot growth. Even though the more vigorous shoot growth did not affect amino acid profiles, ripening was delayed and during some seasons the occurrence of botrytis was enhanced, resulting in wine of lower quality. Excessive N applications at budbreak should thus be avoided, especially for vigorous cultivars. Where vines tend to grow vigorously, a single application of N during the post-harvest period, or incremental

TABLE 6

Effect of application time and source of N on wine quality for Bukettraube.

Treatment [1]	1988/89 Season		1989/90 Season	
	Overall wine quality (%)	Cultivar character (%)	Overall wine quality (%)	Cultivar character (%)
N_0 – Control	48.7 ab	43.2 ab	45.4 a	48.4 a
N_1 – Split applications	59.2 abc	61.1 bc	46.8 a	59.5 ab
N_2 – Single application at budbreak	42.6 a	30.3 a	52.6 a	58.1 ab
N_3 – Single post-harvest application	61.8 bc	69.1 c	48.4 a	55.7 ab
N_4 – Poultry manure as N-source	66.7 c	61.8 bc	54.0 a	65.3 b

[1] See Table 1 for full details of treatments.
[2] Values within columns, followed by the same letter, do not differ significantly ($p \leq 0.05$).

applications (budbreak, fruitset and post-harvest), should be viable options. Applications at fruitset, however, will only be effective if combined with adequate irrigation practices. Even on sandy soil with low organic material annual application of 50 kg N/ha should ensure sufficient assimilable N and acceptable wine quality for grapevines grafted onto Ramsey. At higher application rates, juice composition and wine quality may be affected negatively (Conradie & Saayman, 1989; Spayd *et al.*, 1994).

CONCLUSIONS

An annual application of 50 kg N/ha in the inorganic form appeared to be compatible with a realistic yield and relatively high wine quality for grapevines grown on low-fertility sandy soil in the coastal region of South Africa. Where grapevines received no N, juice still contained more than 400 mg/L of assimilable N, but wine quality was reduced. The observed response, obtained with Ramsey as rootstock, may be different for less vigorous rootstocks. This aspect should be further investigated under South African conditions.

The total N content of leaf blades and petioles at fruitset was a poor predictor of total N in juice at harvest, while NO_3-N in petioles correlated better. Factors such as crop load and climatic conditions between fruitset and harvest, play an important role and different sets of norms will be required for different situations.

Chemical analysis of juice can give a broad indication of eventual wine quality, but high/low quality cannot always be predicted. In numerous fertilisation trials "wine quality" is still defined solely using analytical data. It is essential for all fertilisation trials to include sensory evaluation of experimental wines.

There were indications that the use of an organic N source on sandy soil may lead to wines of slightly higher quality. Even though organic fertilisation is expected to be less advantageous on more clayey soils, this environmentally friendly practice should be investigated further for different cultivars in different areas.

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