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Diurnal and seasonal changes in nitrate reductase activity and nitrogen content of grapevines: Effect of canopy management

by

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S u m m a r y : Diurnal and seasonal *in vivo* nitrate reductase activity (NRA) and nitrogen (N) contents in leaves, berries, and roots and the effect of canopy management were investigated in *Vitis vinifera* L. cv. Cabernet Sauvignon/99 Richter grapevines. Peak NRA in leaves occurred from mid-morning to mid-day. Young leaves had almost the same NRA as mature leaves before berries reached pea size stage, but subsequent to that displayed higher activity. Leaf NRA increased during the post-harvest period. Differences in NRA patterns between leaves conformed with classic source:sink behaviour. Canopy management stimulated nitrate reduction in basal source leaves, most likely through its favourable effect on canopy light microclimate and photosynthetic activity. The NRA in the berries generally increased towards ripeness; treatments affected NRA only slightly. Peak root NRA corresponded to seasonal root growth patterns. In contrast to leaves and berries, NRA in roots increased from the morning to the afternoon. Effect of treatment on root NRA was minor. Leaf and berry N contents declined during the season, whereas reasonably stable concentrations were maintained in the roots. An involvement of NR in the N assimilation and in the energy supply pathways of the grapevine was substantiated. NRA proved to be a good indicator of fluctuations in N assimilation during growth, suggesting its determination to be instrumental in defining the N status and fertilization needs of the grapevine.

K e y w o r d s : grapevine, nitrate reductase, nitrogen, leaves, berries, roots, canopy management.

Tageszeitlich und saisonal bedingte Veränderungen der Nitratreduktase-Aktivität und des Stickstoffgehalts von Weinreben: Der Einfluß gezielter Laubarbeit

Tages- und jahreszeitlich bedingte Veränderungen der Nitratreduktase-Aktivität (NRA) sowie des Stickstoffgehalts von Blättern, Beeren und Wurzeln von *Vitis vinifera* L. (cv. Cabernet Sauvignon auf 99 Richter) wurden in Abhängigkeit von der Laubbehandlung untersucht. Generell traten höchste NR-Aktivitäten im Blatt gegen Mittag auf. Zu Beginn der Vegetationsperiode wurden bei jungen und ausgewachsenen Blättern vergleichbare Werte gemessen; danach waren sie in jungen Geweben höher. Die Blatt-NRA stieg zudem nach der Lese nochmals deutlich an. Die Unterschiede zwischen den Blattstadien spiegeln das erwartete Source-Sink-Muster wider.

Gezielt durchgeführte Laubarbeiten stimulierten die NRA in ausgewachsenen Blättern, vermutlich vor allem durch den positiven Effekt auf die Photosynthese infolge besserer Lichtverhältnisse im Inneren der Laubwand.

Die NRA stieg in den Beeren mit zunehmender Reife an. Die höchsten Werte in den Wurzeln fielen zeitlich mit der Periode des stärksten Wurzelwachstums zusammen. Der Effekt der Laubarbeit war hingegen in beiden Fällen vernachlässigbar gering. Im Gegensatz zu den Verhältnissen in Blättern und Beeren erhöhte sich die NRA der Wurzeln im Tagesverlauf.

Der prozentuale Stickstoffgehalt von Blättern und Beeren nahm im Laufe der Vegetationsperiode ab, während er in den Wurzeln mehr oder weniger konstant war. Es werden Zusammenhänge zwischen der NRA und der N-Assimilation deutlich, so daß die Bestimmung der Enzymaktivität als ein gangbarer Weg zur Erfassung des N-Status und damit des Düngerbedarfs von Weinreben erscheint.

Introduction

It is well known that the nitrogen (N) nutrition of grapevines is important for the stimulation of growth, and the optimization of must composition and fermentation to ensure the production of high quality wines (CHRISTENSEN *et al.* 1994, and references therein; SPAYD *et al.* 1995; VERSINI *et al.* 1995). However, although a number of studies focused on the utilization and partitioning of N at various physiological stages during the growth season, key enzymatic reactions involved in N metabolism of the grapevine have received little attention (PÉREZ and KLIEWER 1978 and 1982; GHISI *et al.* 1984; SCHALLER 1984; PÉREZ and VALDÉS 1989). These studies dealt primarily with leaves and, with the exception of GHISI *et al.* (1984), demonstrated the presence of a cytoplasmically located nitrate reductase (NR) enzyme (NADH-nitrate oxidoreductase, E.C. 1.6.6.1), which is substrate inducible and the first enzyme of nitrate metabolism (BEEVERS and HAGEMAN 1969). It has been shown that shading increased NH_4 and NO_3 levels, but reduced NR activity (NRA) in leaves; petiole nitrate concentration was also inversely related to NRA. KRUEGER and KLIEWER (1995) reported that leaf exposure as well as carbohydrate status and reductant supply increased arginine formation in both leaves and berries. ARAUJO and WILLIAMS (1988) found a linear relationship between vine nitrogen

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content and leaf dry mass and suggested that the leaves are decisive for total vine N content.

Assessments of NRA in grapevines would therefore provide new perspectives on their nutritional status and metabolic competence for N utilization. It would also be a major parameter in determining the productive lifespan of individual leaves, particularly those situated in the interior of the canopy where they are normally exposed to poorer light conditions (SMART *et al.* 1988; HUNTER *et al.* 1995 a). Since NRA indicates the turnover of newly-absorbed nitrogen, monitoring NRA will also contribute to our understanding of nitrogen partitioning and source:sink relationships.

In this study the qualitative NO_3 reducing capacity as well as the N contents of grapevines were followed from berry set to post-harvest. The NRA of young and mature leaves and the effect of canopy management were investigated. Results are discussed in relation to previous work on photosynthetic activity and sugar production patterns (HUNTER *et al.* 1994).

Material and methods

Plant material: Eleven-year-old *Vitis vinifera* cv. Cabernet Sauvignon (clone CS46) vines, grafted onto 99 Richter (clone RY 30), and grown in the Western Cape at Nietvoorbij, Stellenbosch, were used. Vines were spaced 3.0 m x 1.5 m on a Glenrosa soil (Series 13, Kanonkop; MACVICAR *et al.* 1977) and trained to a 1.5 m slanting trellis as described by ZEEMAN (1981). Bud loads of 10 buds per kg cane mass were applied. Vines were irrigated (50 mm) just after pea size and veraison stages, respectively.

Treatments: Experiment I: No treatments were applied. All vines were suckered, i.e. at 30 cm length all shoots not being located on two-bud spurs were removed.

Experiment II: This experiment comprised two treatments, i.e. control and canopy management. The latter was a combination of suckering, shoot positioning, and leaf removal (33 % in the zone opposite and below bunches at berry set and in the remainder of the lower half of the canopy at the pea size stage).

Sampling: Where applicable, the first 3 basal leaves above the upper bunch and the last 6 apical leaves were sampled. The main bunches were harvested, destemmed and a representative berry sample used for analyses. A root sample consisting of all root sizes was obtained by using a soil auger of approximately 7 cm in diameter to a depth of 30 cm randomly at 30 cm distance from the vine trunk. Roots were retrieved by careful washing of the soil.

Experiment I: Sampling of apical and basal leaves took place at 08:00 and at 1.5-h-intervals until 18:00 at berry set, pea size, veraison, and ripeness stages of berry development and at post-harvest (one month after harvest).

Experiment II: Basal leaves, berries and roots were sampled at 10:30 and 15:30 at berry set, pea size, veraison, ripeness and post-harvest stages.

Leaves and bunches were sampled from one shoot on each of 4 vines, whereas roots were sampled from 3 vines, at each sampling time; a composite root sample was used for analysis. All samples were processed immediately.

Nitrate reductase activity (NRA) assay: A modified *in vivo* method as described by HUNTER and VISSER (1986) was used. After removal of leaf veins, leaves were cut into 2 mm^2 discs. Berries and roots were cut into 2 mm wide slices. Representative samples of leaves (0.2 g), berries (1 or 2 g), and roots (1 g) were immediately infiltrated under vacuum in pre-cooled 50 ml Erlenmeyer flasks containing 5 ml 0.1 M KNO_3 and 5 ml 0.1 M phosphate ($Na_2HPO_4 \cdot 12\ H_2O$-KH_2PO_4) buffer at pH 7.5. In controls, KNO_3 was substituted by water. Infiltration of tissue with incubation medium comprised repetitive (5 x 30 s) removal of oxygen by vacuum and replacing it with N_2. After infiltration, N_2 was bubbled into the incubation medium for 60 s. Flasks were then sealed with rubber stoppers, wrapped in aluminium foil and incubated in a water bath at 40 °C with gentle shaking for 1 h. After incubation flasks were vortexed for 10 s and 1 ml aliquots removed for nitrite determination. Nitrite formed was estimated by adding 1 cm^3 1 % (w/v) sulphanilamide in 1.75 M HCl, 1 ml 0.01 % (w/v) N-(1-naphthyl)ethylenediamine dihydrochloride and 5 ml H_2O. Absorbance was read at 540 nm with a LKB UV/VIS spectrophotometer after 30 min. The NRA was expressed as nmol nitrite produced per gram fresh tissue per hour after NRA of control treatments was subtracted.

Total nitrogen content: All samples taken in Experiment II were processed to determine total nitrogen content. Samples were stored at -20 °C, freeze-dried, and ground (20 mesh) prior to digestion by the standard Kjeldahl procedure and analysed by an Auto-Analyzer (Technicon).

Experimental design and statistical analyses: A completely randomised experimental design was used. Treatments were applied for two consecutive years. A one-way analysis of variance was performed and Student's t-test used to determine significant differences between treatment means.

Results and Discussion

Nitrate reductase activity in grapevine leaves generally peaked during the mid-morning to mid-day period, whereafter it decreased to low levels in the late afternoon (Figure). This corresponds to the diurnal rhythm of NRA found by SCHALLER (1984) for Riesling vines and by HUBER *et al.* (1992) for spinach grown under controlled conditions. These results as well as the difference in NRA between young and mature leaves during the growth season correlate well with the respective rates of photosynthetic activity (HUNTER *et al.* 1994). Young, expanding leaves displayed similar or slightly lower activity than mature, exporting leaves early in the season, but higher activity from veraison onwards. The seasonal pattern of NRA parallelled that of sucrose (HUNTER *et al.* 1994) and starch

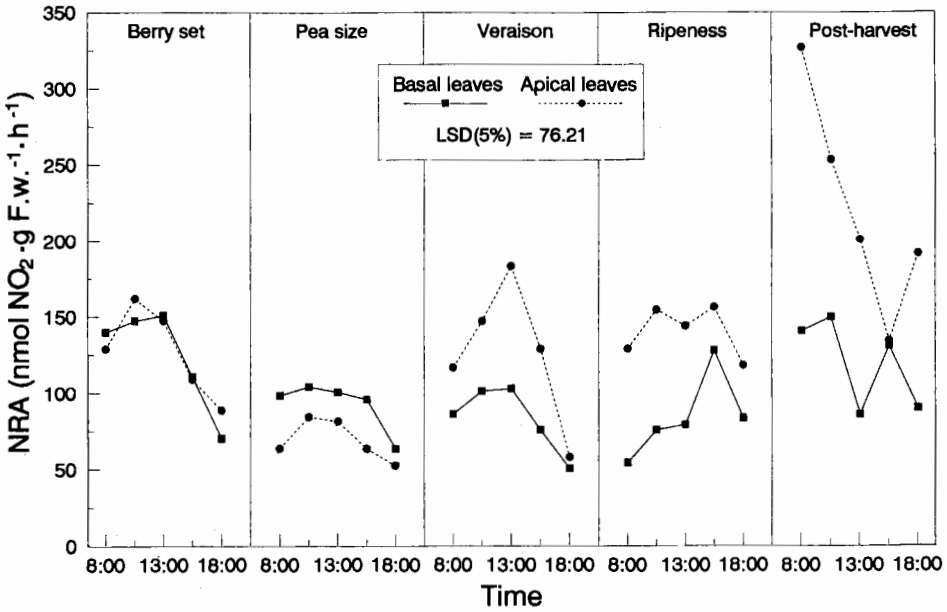

Figure: Diurnal nitrate reductase activity (NRA) in basal and apical leaves of Cabernet Sauvignon/99 Richter grapevines at different stages of development.

(HUNTER *et al.* 1995 b) accumulation. In particular, the increase at the post-harvest stage appears to favour annual N reserve accumulation (Figure). There is conclusive evidence that the availability of carbohydrates acting as a prime source of energy enhances the rate of nitrate reduction (KLEPPER *et al.* 1971; HUBER *et al.* 1992, and references therein).

Table 1

Morning (10:30) and afternoon (15:30) nitrate reductase activities in basal leaves, berries and roots of Cabernet Sauvignon/99 Richter grapevines at different developmental stages and the effect of canopy management. The sampling method prevented normal statistical analyses on the values of roots

Developmental stage	Morning values (nmole NO_2·g F.w.h^{-1})		Afternoon values (nmole NO_2·g F.w.h^{-1})	
	Control	Treated	Control	Treated
	LEAVES			
Berry set	136.0	166.3	89.0	140.7
Pea size	65.0	107.4	50.7	67.5
Veraison	117.0	113.2	88.7	81.0
Ripeness	67.8	102.2	56.5	62.8
Post-harvest	136.3	152.5	144.2	190.5
LSD (5 %)	43.34			
	BERRIES			
Berry set	4.5	6.0	2.0	2.5
Pea size	3.5	3.5	1.0	2.3
Veraison	19.4	11.7	16.4	16.2
Ripeness	24.2	24.8	18.1	16.7
LSD (5 %)	5.36			
	ROOTS			
Berry set	24.4	35.4	44.2	36.1
Pea size	15.2	19.1	18.7	23.7
Veraison	12.3	11.6	22.0	19.6
Ripeness	29.5	23.7	30.6	27.0
Post-harvest	18.5	18.5	24.6	21.1

In young, immature leaves photosynthates are deposited into local growth and development (HUNTER and VISSER 1988 a). HUBER *et al.* (1992) suggested that NR plays an important role in the production of amino acids during spinach leaf expansion. The importance of amino acids, proteins, and nucleic acids for growth and the generally higher rate of nitrate reduction in young leaves during the latter part of the season are consistent with the classic concept of source:sink differences between young and mature leaves. Furthermore, under the conditions of our experiment, young, apical leaves were exposed to a higher sunlight level than basal leaves, particularly later in the season (HUNTER and VISSER 1988 b). Sunlight is known to activate the NR enzyme complex (PÉREZ and KLIEWER 1982). Nevertheless, constant high NRA in basal leaves indicates a significant role of source leaves in nitrate reduction and amino acid export.

In general, NRA in basal leaves was stimulated by canopy management and the concomitant changes in source:sink relationships (Tab. 1). It can reasonably be assumed that the higher interior canopy light intensities and photosynthetic activities of treated vines (HUNTER *et al.* 1995 a) were instrumental in increasing enzyme activity. KLEPPER *et al.* (1971) proposed that sugars that migrate from the chloroplast to the cytoplasm are the main source of energy for nitrate reduction in leaves and that the oxidation of glyceraldehyde-3-phosphate was ultimately the *in vivo* source of NADH. HUBER *et al.* (1992) demonstrated that the nitrate reducing (NR) and sucrose synthesizing (sucrose-phosphate synthase) enzymes share common features in that both are light-activated, have definite photoperiodical patterns, and are regulated by end products of photosynthesis (*inter alia* amino acids and sucrose), and are therefore most likely coordinated with one another and with the rate of photosynthesis. In contrast to ASLAM and HUFFAKER (1984) who do not consider light as a prerequisite for NR induction, others proposed that light has a direct effect on nitrate assimilation because it stimulates the

uptake of nitrate, promotes the transfer of nitrate from storage to metabolic pools, induces synthesis of NR, and activates pre-existing inactive NR (NAIK *et al.* 1982). SMART *et al.* (1988) found that red light caused increased nitrite accumulation in Cabernet Sauvignon leaves. In soybean, red light was also found to increase the levels of NR and carbohydrate accumulation (BARRO *et al.* 1989). Relating the lack of nitrate metabolism to inefficient assimilation at low light intensity, PÉREZ and KLIEWER (1982) concluded that exposure of leaves to saturating or higher light levels results in the utilization of high amounts of nitrate by grapevines, which in the long term affects productivity. This may have been one of the reasons for the continued and/or enhanced performance found in partially defoliated grapevines in previous studies (HUNTER *et al.* 1995 a). In both, treated and untreated plants, NRA generally decreased between morning and afternoon. Basic metabolic processes therefore do not seem to be changed by canopy management.

Nitrate reductase activity was low in green berries, but increased substantially during ripening (Tab. 1). This corresponds to the influx of sugar and is in general agreement with the energy requirements for NRA as discussed before. The pattern also parallels arginine accumulation in the grapes (CONRADIE and SAAYMAN 1989). Berry arginine content is commonly suggested to be an indicator of the nitrogen status of the plant (KLIEWER and COOK 1974; SCHALLER *et al.* 1989; KRUEGER and KLIEWER 1995). As in leaves, NRA of berries decreased from the morning to the afternoon. Differences between treated and control plants were minor. Considering the improved light conditions in the bunch zone created by applying canopy management (HUNTER *et al.* 1995 a) as well as the differences in NRA observed in leaves, higher berry NRA was expected for treated vines. However, increased amino acid formation in treated leaves and thus transport from leaves to berries may have reduced the induction of nitrate reductase in the berries. According to GLAD *et al.* (1992) glutamine, and to a lesser extent proline, are the amino acids mainly transported in the phloem. The NRA of the berries is therefore not necessarily an indicator of their amino acid content or total nitrogen status.

Root NRA fluctuated during the season, being highest at the time of berry set, lowest at pea size/veraison, and high again at ripeness (Tab. 1). Apparently responding to the influx of nitrate, peak activities corresponded to seasonal root growth rates as reported for Colombar/99 Richter vines (VAN ZYL 1984). In Cabernet Sauvignon, another late ripening cultivar, root growth activity had apparently already started at ripeness. In contrast to leaves and berries, NRA in the roots increased between morning and afternoon, presumably reacting to the increase in soil temperature and thus nitrate uptake (CONRADIE 1991) during the day. As for berries, treated and control plants differed only slightly in root NRA.

The seasonal patterns of N accumulation in the different tissues correspond to that found for photosynthetic activity (HUNTER *et al.* 1994) (Tab. 2). A linear relationship between leaf nitrogen content and CO_2 assimilation rate

Table 2

Morning (10:30) and afternoon (15:30) nitrogen contents in basal leaves, berries and roots of Cabernet Sauvignon/99 Richter grapevines at different developmental stages and the effect of canopy management

Developmental stage	Morning values (N %)		Afternoon values (N %)	
	Control	Treated	Control	Treated
	LEAVES			
Berry set	2.07	2.25	2.17	2.13
Pea size	1.55	1.72	1.57	1.73
Veraison	1.34	1.43	1.27	1.35
Ripeness	0.96	1.15	0.90	1.05
Post-harvest	1.09	1.04	1.06	1.14
LSD (5 %)	0.214			
	BERRIES			
Berry set	1.56[1]		1.70[1]	
Pea size	1.24	1.23	1.14	1.20
Veraison	0.65	0.69	0.71	0.68
Ripeness	0.36	0.42	0.36	0.39
LSD (5 %)	0.197			
	ROOTS			
Berry set	0.67	0.71	1.13	0.57
Pea size	0.86	0.73	0.67	0.74
Veraison	0.87	0.61	0.83	0.73
Ripeness	0.48	0.47	0.71	0.67
Post-harvest	0.70	0.61	0.70	0.73
LSD (5 %)	0.134			

[1] Combined samples.

during the post-harvest period has previously been demonstrated and indicates that leaf nitrogen content may be used as a measure of the photosynthetic capacity of grapevines during this period (WILLIAMS and SMITH 1985). The decrease in N content in Cabernet Sauvignon towards ripeness parallels the results of CONRADIE (1981), GHISI *et al.* (1984) and ARAUJO and WILLIAMS (1988) for Chenin blanc, Merlot and Thompson Seedless, respectively. A high berry N content during the first part of the growth season (until pea size stage) matches cell division and growth of the berries, whereas the low N contents at and after veraison must have resulted from dilution due to the increase in berry size. Similar N fluctuations in berries were found by PATIL and GUPTA (1973). Berry N contents are directly inverse to their NRA. Although roots obviously have a strong ability to regulate carbohydrate import which supports high levels of NRA during peak root growth periods, the virtually stable N contents in roots indicate an effective N influx/efflux control system in these organs. Since carbohydrates are translocated from the leaves to the roots, KLEPPER *et al.* (1971) suggested that the process for NADH generation and utilization in non-photosynthetic tissue is similar to that operating in leaves (cf. also HUNTER and VISSER 1987).

It was expected that in the control vines, N contents would reflect the bigger canopy size and the concomitant

increased transpiration rates. However, any primary response to the increase in canopy size seemed to be counterbalanced by the effect of the shaded canopies (in the case of control vines) or the improved conditions for N utilization (due to careful canopy manipulation).

Conclusions

Seasonal fluctuations of NRA in grapevine leaves and roots corresponded to periods of maximal root growth, suggesting that determination of NRA in leaves (and possibly petioles) provides valuable information on nitrate assimilation, which in turn can be used for the timing as well as the refinement of N fertilization. Evidently, N supplementation should focus on the periods of berry set, veraison, and post-harvest to ensure effective N uptake and utilization. Monitoring NRA as well as total N content allows a distinction between newly absorbed N and N already incorporated into amino acids and proteins. Similarities to previously described patterns of grapevine photosynthesis and carbohydrate accumulation are obvious. Nitrate reductase is actively involved in nitrogen assimilation of the grapevine. It plays a prominent role in the allocation of photosynthetic energy by mediating N distribution. Clearly, the basic requirements of the enzyme (such as NO_3 availability, energy charge and adequate sun exposure of the leaves) must primarily be satisfied and the seasonal NRA level taken into account, before optimal development of the plant and ultimate expression of the full qualitative potential of the grape can be expected.

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References

ARAUJO, F. J.; WILLIAMS, L. E.; 1988: Dry matter and nitrogen partitioning and root growth of young field-grown Thompson Seedless grapevines. Vitis **27**, 21-32.

ASLAM, M.; HUFFAKER, R. C.; 1984: Dependency of nitrate reduction on soluble carbohydrates in primary leaves of barley under aerobic conditions. Plant Physiol. **75**, 623-628.

BARRO, F.; DE LA HABA, P.; MALDONADO, J. M.; FONTES, A. G.; 1989: Effect of light quality on growth, contents of carbohydrates, protein and pigments, and nitrate reductase activity in soybean plants. J. Plant Physiol. **134**, 586-591.

BEEVERS, L.; HAGEMAN, R. H.; 1969: Nitrate reduction in higher plants. Ann. Rev. Plant Physiol. **20**, 495-522.

CHRISTENSEN, L. P.; BIANCHI, M. L.; PEACOCK, W. L.; HIRSCHFELT, D. J.; 1994: Effect of nitrogen fertilizer timing and rate on inorganic nitrogen status, fruit composition, and yield of grapevines. Amer. J. Enol. Viticult. **45**, 377-387.

CONRADIE, W. J.; 1981: Nutrient consumption by Chenin blanc grown in sand culture and seasonal changes in the chemical composition of leaf blades and petioles. S. Afr. J. Enol. Viticult. **2**, 15-18.

- -; 1991: Translocation and storage of nitrogen by grapevines as affected by time of application. In: RANTZ, J. M. (Ed.): Proc. Intern. Symp. on Nitrogen in Grapes and Wine, 32-42, 18-19 June 1991, Seattle, USA.

- -; SAAYMAN, D.; 1989: Effect of long-term nitrogen, phosphorus, and potassium fertilization on Chenin blanc vines. II. Leaf analyses and grape composition. Amer. J. Enol. Viticult. **40**, 91-98.

GHISI, R.; IANNINI, B.; PASSERA, C.; 1984: Changes in the activities of enzymes involved in nitrogen and sulphur assimilation during leaf and berry development of *Vitis vinifera*. Vitis **23**, 257-267.

GLAD, C.; REGNARD, J. L.; QUEROU, Y.; BRUN, O.; MOROT-GAUDRY, J. F.; 1992: Phloem sap exudates as a criterion for sink strength appreciation in *Vitis vinifera* cv. Pinot noir grapevines. Vitis **31**, 131-138.

HUBER, S. C.; HUBER, J. L.; CAMPBELL, W. H.; REDINBAUGH, M. G.; 1992: Comparative studies of the light modulation of nitrate reductase and sucrose-phosphate synthase activities in spinach leaves. Plant Physiol. **100**, 706-712.

HUNTER, J. J.; RUFFNER, H. P.; VOLSCHENK, C. G.; LE ROUX, D. J.; 1995 a: Partial defoliation of *Vitis vinifera* L. cv. Cabernet Sauvignon/99 Richter: Effect on root growth, canopy efficiency, grape composition, and wine quality. Amer. J. Enol. Viticult. **46**, 306-314.

- -; - -; - -; 1995 b: Starch concentrations in grapevine leaves, berries and roots and the effect of canopy management. S. Afr. J. Enol. Viticult. **16**, 35-40.

- -; SKRIVAN, R.; RUFFNER, H. P.; 1994: Diurnal and seasonal physiological changes in leaves of *Vitis vinifera* L.: CO_2 assimilation rates, sugar levels and sucrolytic enzyme activity. Vitis **33**, 189-195.

- -; VISSER, J. H.; 1986: The nitrate reductase activity (NRA) of some South African parasitic flowering plants and their hosts. S. Afr. J. Bot. **52**, 81-84.

- -; - -; 1987: Sources of energy and inducement of nitrate reductase activity (NRA) in a host: root parasitic relationship. S. Afr. J. Bot. **53**, 1-4.

- -; - -; 1988 a: Distribution of ^{14}C-photosynthetate in the shoot of *Vitis vinifera* L. cv. Cabernet Sauvignon. I. The effect of leaf position and developmental stage of the vine. S. Afr. J. Enol. Viticult. **9**, 3-9.

- -; - -; 1988 b: The effect of partial defoliation, leaf position and developmental stage of the vine on the photosynthetic activity of *Vitis vinifera* L. cv. Cabernet Sauvignon. S. Afr. J. Enol. Viticult. **9** (2), 9-15.

KLEPPER, L.; FLESHER, D.; HAGEMAN, R. H.; 1971: Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. Plant Physiol. **48**, 580-590.

KLIEWER, W. M.; COOK, J. A.; 1974: Arginine levels in grape canes and fruits as indicators of nitrogen status of vineyards. Amer. J. Enol. Viticult. **25**, 111-118.

KRUEGER, R.; KLIEWER, W. M.; 1995: Arginine synthesis in grapevine leaves and berries: Diurnal and seasonal patterns, environmental and physiological influences. Amer. J. Enol. Viticult. **46**, 37-42.

MACVICAR, C. N.; SOIL SURVEY STAFF; 1977: Soil classification. A binomial system for South Africa. Scientific Pamphlet 390. Department of Agricultural Development, Pretoria, RSA.

NAIK, M. S.; ABROL, Y. P.; NAIR T. V. R.; RAMARAO, C. S.; 1982: Nitrate assimilation - its regulation and relationship to reduced nitrogen in higher plants. Phytochemistry **21**, 495-504.

PATIL, A. V.; GUPTA, P. K.; 1973: Sugar, acid and nitrogen in the developing berries of some grape varieties. Vitis **12**, 214-221.

PÉREZ, H. J.; VALDÉS, L. M.; 1989: Effect of nitrogen fertilization and shading on bud fruitfulness and bud necrosis in cv Sultanina (Thompson Seedless). Ciencia e Investigación Agraria **16**, 89-98.

PÉREZ, J. R.; KLIEWER, W. M. 1978: Nitrate reduction in leaves of grapevine and other fruit trees. J. Amer. Soc. Hort. Sci. **103**, 246-250.

- -; - -; 1982: Influence of light regime and nitrate fertilization on nitrate reductase activity and concentrations of nitrate and arginine in tissues of three cultivars of grapevines. Amer. J. Enol. Viticult. **33**, 86-93.

SCHALLER, K.; 1984: Untersuchungen zur Nitratreduktase-Aktivität in Reben. Mitt. Klosterneuburg **34**, 235-240.

- -; LÖHNERTZ, O.; GEIBEN, R.; BREIT, N.; 1989: N-Stoffwechsel von Reben I. Mitteilung: N- und Arginindynamik im Holzkörper der Sorte Müller-Thurgau im Verlaufe einer Vegetationsperiode. Wein-Wiss. **44**, 91-101.

SMART, R. E.; SMITH, S. M.; WINCHESTER, R. V.; 1988: Light quality and quantity effects on fruit ripening for Cabernet Sauvignon. Amer. J. Enol. Viticult. **39**, 250-258.

SPAYD, S. E.; NAGEL, C. W.; EDWARDS, C. G.; 1995: Yeast growth in Riesling juice as affected by vineyard nitrogen fertilization. Amer. J. Enol. Viticult. **46**, 49-55.

VAN ZYL, J. L. 1984: Response of Colombar grapevines to irrigation as regards quality aspects and growth. S. Afr. J. Enol. Viticult. **5**, 19-28.

VERSINI, G.; LUNELLI, M.; SEPPI, A.; DALLA SERRA, A.; VOLONTERIO, G.; 1995: Aspetti connessi alla produzione di vini base-spumante da Chardonnay e Pinot nero. Enotecnico (4), 49-65.

WILLIAMS, L. E.; SMITH, R. J.; 1985: Net CO_2 assimilation rate and nitrogen content of grape leaves subsequent to fruit harvest. J. Amer. Soc. Hort. Sci. **110**, 846-850.

ZEEMAN, A. S.; 1981: Oplei. In: BURGER, J. D.; DEIST, J. (Eds.): Wingerdbou in Suid-Afrika, 185-201. Nietvoorbij, Stellenbosch.

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