Vitis 34 (1), 19–26 (1995)

Carbon and nitrogen partitioning in *Vitis vinifera* L.: Responses to nitrogen supply and limiting irradiance

by

M. KELLER[1,2], B. HESS[2], H. SCHWAGER[1], H. SCHÄRER[1] and W. KOBLET[1]

[1] Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture, Wädenswil, Switzerland
[2] Swiss Federal Institute of Technology, Department of Plant Science, Zürich, Switzerland

S u m m a r y : Potted *Vitis vinifera* L. plants were grown under controlled environmental conditions at five different levels of nitrogen (0, 1, 5, 10, 100 mM NH_4NO_3) in combination with two different levels of irradiance (photon flux densities: 30 and 140 µmol · m^{-2} · s^{-1} PAR) during bloom. Elevated N supply increased available N (particularly NO_3^-), K, Ca and Mg, and reduced P in the soil. Soil-NO_3^- and K were higher in the lower light regime, but NH_4^+ and other nutrients were not influenced by irradiance. The concentration of total N in the xylem sap increased as N supply was increased, although there was no further rise above intermediate soil-N levels. NO_3^- was the principal xylem solute, in particular under severe light restriction and high N availability. In the lower light regime, only traces of organic N could be detected in the xylem sap, whereas in the higher light treatment, glutamine and glutamate increased with increasing N application level. Light limitation reduced the concentrations of P, K and Mg in the xylem sap by about 50 %, but no response to N supply could be observed.

There was a strong positive relationship between N availability and N concentration in all plant parts, while the effect on C content was minor and depended on the type and physiological age of the tissue. The amounts of total N per vine were not affected by the light treatments, although low-light stress increased N concentrations in the dry matter of the annual organs by 34–86 %. By contrast, low light led to a slight decrease of the C concentration in the annual plant parts. In the higher light regime, non-structural carbohydrates in the permanent parts of the vine declined as N availability increased. Under severe light restriction, however, the C reserve fraction was depleted and was not altered by N supply, indicating that reserves had been remobilized to support maintenance and growth processes, in order to guarantee survival of the vine.

K e y w o r d s : light, nitrogen, carbon, source, sink, bloom, stress physiology, xylem solutes, reserves.

Introduction

For early growth, grapevines utilize exclusively retranslocated reserves from their permanent parts. Currently assimilated carbon (C) from the basal leaves increasingly contributes to shoot growth, while translocation of photosynthates to the lower parts of the vine begins around the 10-leaf stage and increases rapidly at bloom (KOBLET 1969, YANG and HORI 1979). According to YANG *et al.* (1980), retranslocation of reserves from the perennial parts typically is discontinued during bloom, suggesting that the main leaves are the only source organs at this time. From bud break to the end of bloom, the vine is also heavily dependent on nitrogen (N) reserves stored in the permanent structure (CONRADIE 1991, WILLIAMS 1991). Ensuing the C supply from the shoots, N uptake from the soil increases about two weeks before flowering and reaches a maximum just after bloom (LÖHNERTZ 1988). Uptake until the end of bloom constitutes less than 30 % of the seasonal demand, although the leaves may accumulate up to 60 % of their seasonal N requirement (reviewed by CONRADIE 1991). Both C and N reserves in wood and roots therefore reach a minimum by bloom time (STOEV *et al.* 1966, WINKLER *et al.* 1974, SCHALLER *et al.* 1989).

Recently, GEIGER and SERVAITES (1991) suggested that sucrose and starch in source leaves and permanent organs may serve as buffer reserves by providing a temporary supply of C to be mobilized when the supply of photosynthates becomes limited. The partitioning of newly fixed C or reserves is determined by the relative sink priority of the different organs (Ho 1988).The flowering period is crucial for reproductive growth, since stress situations impairing photosynthesis may result in a shortage in C translocation to the flower clusters, which are low-priority sinks (KELLER and KOBLET 1994). Current fertilization recommendations tend to split N applications or delay them until bloom or later to optimize the availability during the critical period and minimize the leaching and denitrification potential (PEACOCK *et al.* 1989, GOLDSPINK and GORDON 1991, PERRET 1993). However, the impact of adverse weather conditions on the grapevine's response to soil N level is not clear, although in higher plants the rate of N uptake often exceeds the rate of N assimilation (MENGEL 1985). The purpose of this study was therefore, to evaluate N uptake, transport and distribution among plant parts in response to N supply under light-stress conditions. We were also interested in the effect of N on the vines' capability to remobilize carbohydrate reserves from the permanent parts during bloom to compensate for the leaves' restricted source-capacity.

Correspondence to: M. KELLER, Cornell University, Food Research Laboratory, Geneva, NY 14456-0462, USA.

Materials and methods

Growth and experimental conditions: Two-year-old *Vitis vinifera* L. grapevines (cv. Müller-Thurgau grafted on SO 4) were grown in 18-liter pots on sandy loam soil under field climatic conditions without fertilization in 1990. On February 1, 1991, they were placed in two identical rooms of a controlled environment facility (phytotron). The vines were irrigated to capacity with tap water (mg l^{-1}: 1.4 N, 0.0 P, 0.7 K, 34.1 Ca, 57.8 Mg) at 1-week intervals, before treatments were imposed. By this time, soil analysis revealed 25.2 ppm N, 4.5 ppm P, 37 ppm K, 120 ppm Ca, 4.2 ppm Mg (available fraction) or 38 ppm P, 164 ppm K, 9380 ppm Ca, 232 ppm Mg (reserve fraction). The N treatments were 0 (N0), 1 (N1), 5 (N5), 10 (N10), 100 (N100) mM NH_4NO_3 in 2 l of distilled H_2O, applied once a week over a 4-week period, beginning on April 15. This amounted to a total of 0, 224, 1120, 2240 and 22,400 mg N per plant, respectively. Two light regimes were imposed in the 2 separate phytotron rooms on April 26 until May 13 (bloom period), for simulation of moderate (treatment ML: photon flux density 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation, PAR) and heavy (treatment LL: 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR) densities of cloud covers. Both phytotron chambers were run with an 8 h photoperiod at 20/11°C day/night temperature and 58/75 % day/night relative humidity. The experimental conditions were described in more detail in a previous publication (KELLER and KOBLET 1994). Three single-plant replications were used per treatment combination. All data were subjected to Bartlett's test to check homogeneity of variance and subsequently to analysis of variance, F-test and Duncan's multiple range test. Selected parameters were examined using correlation and polynomial regression analysis. All statistical tests were performed on WIDAS (Wissenschaftlich Integriertes Daten-Auswertungs-System, MSI Dr. Wälti AG, Switzerland).

Xylem sap collection, xylem solute and soil analysis: We obtained xylem sap non-destructively from all vines 4 times during the course of the experiment (April 25, May 1, 5 and 10). For this purpose, a small hole was bored in the trunk above the soil surface, and xylem sap was extracted with a hypodermic needle (outside diameter 1.2 mm) into a 25-ml glass vial by application of a mild vacuum (Fig. 1) over a 24-h period. The samples were frozen at -18 °C for later analysis

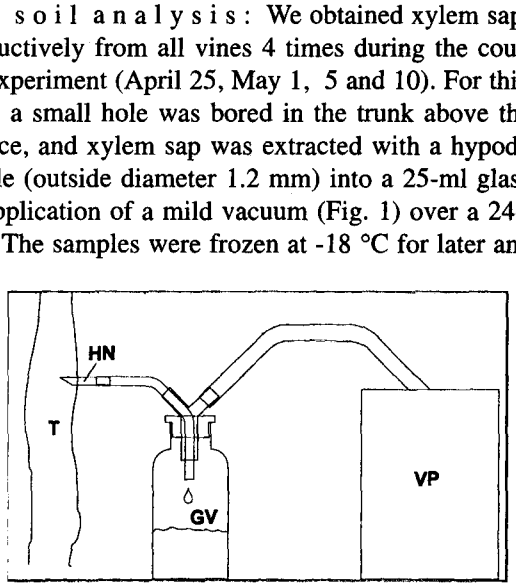

Fig. 1: Non-destructive vacuum extraction of xylem sap from the trunk (**T**) of grapevines. (**HN**) hypodermic needle, (**GV**) glass vial, (**VP**) vacuum pump.

of xylem solutes. For amino acid analysis, samples were thawed and injected into a Stagroma HPLC system (Stagroma AG, Wallisellen, Switzerland) using OPA post column derivatisation. The contents of NO_3^-, NH_4^+, PO_4^{3-} were determined photometrically and K^+ and Mg^{2+} by atomic absorption spectrophotometry, according to the methods described by KÜNSCH *et al.* (1977). Insufficient sample quantities precluded the analysis of Ca^{2+}. For comparison, soil samples were taken before and after imposition of the experimentaltreatments. First, NO_3^- and NH_4^+ were measured in the wet soil. Available and reserve nutrients were then extracted from the oven dried (30°C) soil using 1 : 5 water extract (available fraction) and 1 : 5 NH_4-ac-EDTA extract (pH 4.65) (reserve fraction), respectively and analyzed in the same manner as xylem sap.

Tissue analysis: On May 13, the vines were sampled and separated into roots, trunk, main shoots, lateral shoots, main leaves, lateral leaves and inflorescences. All leaves consisted of blade and petiole. The main leaves were further divided into young (# 1 through 4 from the shoot apex), medium (# 5 through 9) and old (# 10 and older) leaves, while there were never more than 3 leaves per lateral shoot. All organs were oven dried at 65 °C and pulverized in a Cyclotec sample-mill (Tecator Instrumenten Gesellschaft, Zürich, Switzerland). For each plant part, the concentrations of C and N were determined in 5 mg dry tissue samples, using a Heraeus elementary analyzer CHN-O-RAPID (Heraeus AG, Zürich, Switzerland). For the analysis of non-structural carbohydrates, 200 mg root and wood samples, respectively, were utilized. Soluble sugars and starch were extracted and quantified using the method described by CANDOLFI-VASCONCELOS and KOBLET (1990), but 80 % ethanol was used instead of 70 %, and the extracts were centrifuged at 3000 *g* for 10 min.

Results

The concentrations of NO_3^- in the soil exceeded those of NH_4^+ by 4-fold in the low-N treatments to over 20-fold at high N application rates (Fig. 2). Up to N10, soil NH_4^+ remained constant. A strong increase was detectable only at N100. High N fertilization rates slightly decreased the soil-pH from 8.2 in the N0 pots to 7.8 in the N100 pots, in either light regime. Soil NO_3^- and K were lower in ML compared to LL, while NH_4^+, P, Ca and Mg (available fraction) were similar in the two light regimes. In the soil's reserve fraction, no treatment effects became apparent (data not shown). Nevertheless, regression analysis indicated close relationships between N application level and all soil nutrients examined irrespective of the light regime (Tab. 1). Heavy N fertilization increased the availability of K, Ca and Mg and decreased the availability of P in the soil. In the H_2O extract of the low-N pots, however, the concentrations of all nutrients were similar to the initial contents before the treatments were imposed.

The combined effects of a variety of N supply levels and limiting light conditions on xylem solutes are illustrated in Fig. 3. The values displayed are the means of 4

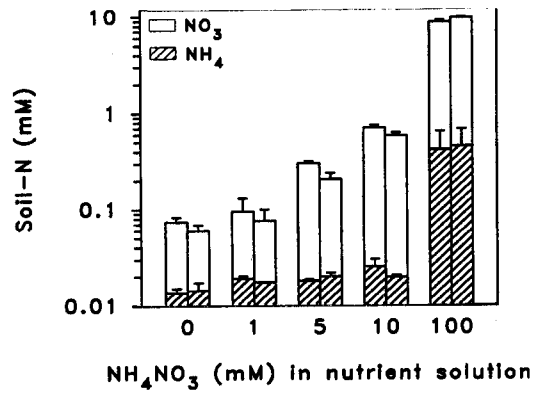

Fig. 2: Effect of irradiance and N application during bloom on soil N in the phytotron at the end of the flowering period. Left bar of each pair: treatment LL, 30 µmol · m^{-2} · s^{-1} PAR; right bar: treatment ML, 140 µmol · m^{-2} · s^{-1} PAR. Values are means ± SE (n = 3).

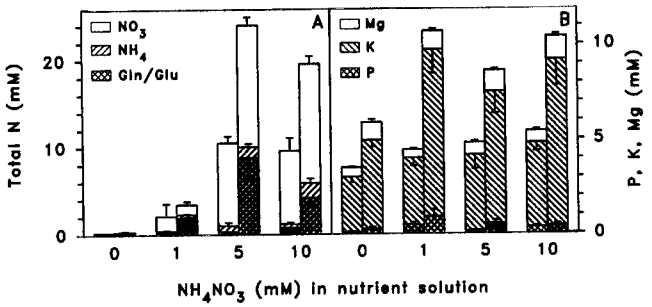

Fig. 3: Effect of irradiance and N application during bloom on xylem sap composition of potted grapevines in the phytotron. Concentrations of organic and inorganic N (**A**) and several macronutrients (**B**). [Left and right bar of each pair: see Fig. 2]. Values are means ± SE (n = 4 to 6).

Table 1

Regression equations for several soil nutrients (y) in the H_2O extract as a function of N application level (x = [mM NH_4NO_3]), 21 d after the first application. The data of 2 light levels are pooled (n = 30), since they did not differ significantly

nutrient (y)	regression equation	r (P < 5 %)
NO_3^- (µM)	$y = 0.348\ x^{\wedge} + 52.93\ x + 24.167$	0.997
NH_4^+ (µM)	$y = 0.038\ x^{\wedge} + 0.410\ x + 15.259$	0.762
N (ppm)	$y = 0.022\ x^{\wedge} + 4.405\ x + 6.226$	0.999
P (ppm)	$y = 0.001\ x^{\wedge} - 0.137\ x + 4.380$	0.880
K (ppm)	$y = 0.009\ x^{\wedge} - 0.482\ x + 25.169$	0.847
Ca (ppm)	$y = 0.044\ x^{\wedge} + 3.811\ x + 108.726$	0.988
Mg (ppm)	$y = 0.001\ x^{\wedge} + 0.183\ x + 9.721$	0.975

consecutive extraction periods on the same plants. The N100 data are missing, because no xylem sap could be extracted at this extremely high N level (compare KELLER and KOBLET 1994). Apart from this, sample volumes obtained using our vacuum technique were comparable in all treatments. Increasing N supply enhanced total xylem N concentration, but there was no further increase above N5, regardless of irradiance (Fig. 3: A), while a significant rise in soil N could only be observed above N1. These data indicate that up to N1, the vines' N demand was higher than supply. As N supply increased, the concentration of inorganic N in the xylem sap rose, and there was a positive correlation between NO_3^- and NH_4^+ ($r = 0.49$, $P < 5\%$), regardless of the light regime. The concentration of total N in the xylem was lower in the LL regime than in ML. This effect was mainly caused by the differences in or-

Table 2

Effect of irradiance and N application during bloom on N concentration (% dry weight) in various organs of potted grapevines in the phytotron. Values are means ± SE (n = 3). Within a column section, means followed by the same letter are not significantly different at P < 5 %

NH_4NO_3	old leaves [a]	young leaves [b]	lateral leaves	inflorescences	main shoots	lateral shoots	trunk	roots
30 µmol · m^{-2} · s^{-1} PAR (treatment LL)								
0 mM	2.60±0.21 c	4.39±0.24 c	3.10±0.28 c	2.05±0.16 b	0.91±0.10 d	2.26±0.14 c	0.58±0.01 b	0.94±0.05 c
1 mM	3.11±0.16 bc	5.43±0.08 b	3.89±0.12 b	2.44±0.08 b	1.16±0.06 c	2.63±0.27 bc	0.60±0.05 b	1.15±0.11 bc
5 mM	3.27±0.15 ab	5.76±0.07 ab	4.41±0.09 ab	2.60±0.15 b	1.31±0.05 bc	2.63±0.21 bc	0.66±0.01 b	1.33±0.10 b
10 mM	3.29±0.17 ab	6.23±0.30 a	4.90±0.27 a	2.54±0.13 b	1.43±0.09 b	2.94±0.11 b	0.64±0.01 b	1.20±0.07 b
100 mM	3.84±0.22 a	5.34±0.07 b	5.02±0.18 a	3.20±0.27 a	2.17±0.09 a	3.55±0.20 a	0.85±0.02 a	1.82±0.06 a
x [c]	3.22±0.13 **	5.45±0.18 **	4.26±0.20 **	2.57±0.12 **	1.40±0.12 **	2.80±0.14 **	0.67±0.03 *	1.29±0.08 n.s.
140 µmol · m^{-2} · s^{-1} PAR (treatment ML)								
0 mM	1.77±0.10 c	1.78±0.13 d	1.67±0.14 c	1.29±0.12 c	0.55±0.04 e	1.68±0.16 b	0.54±0.07 c	0.86±0.03 c
1 mM	1.78±0.05 c	2.39±0.06 c	1.91±0.05 c	1.43±0.01 c	0.74±0.05 d	1.70±0.04 b	0.65±0.01 b	0.94±0.03 c
5 mM	2.41±0.12 b	3.64±0.30 b	3.05±0.10 b	1.90±0.07 b	1.04±0.06 c	2.15±0.21 b	0.74±0.01 b	1.32±0.04 b
10 mM	2.51±0.05 b	4.05±0.05 ab	3.27±0.11 b	2.03±0.08 b	1.18±0.05 b	2.08±0.18 b	0.75±0.02 b	1.29±0.02 b
100 mM	3.14±0.05 a	4.30±0.09 a	3.77±0.19 a	2.67±0.18 a	1.54±0.04 a	2.77±0.02 a	0.88±0.02 a	2.13±0.08 a
x	2.32±0.14	3.23±0.27	2.74±0.22	1.86±0.14	1.01±0.09	2.08±0.12	0.71±0.03	1.31±0.12

[a] leaves # 10 and older from the shoot apex [b] leaves # 1 through 4 from the shoot apex

[c] Means between light regimes differ at ** P < 1%, * P < 5% or n.s. do not differ significantly

Table 3

Effect of irradiance and N application during bloom on C concentration (% dry weight) in various organs of potted grapevines in the phytotron. Values are means ± SE (n = 3). Within a column section, means followed by the same letter are not significantly different at P < 5%

NH_4NO_3	old leaves [a]	young leaves [b]	lateral leaves	inflorescences	main shoots	lateral shoots	trunk	roots
30 μmol · m^{-2} s^{-1} PAR (treatment LL)								
0 mM	43.48±0.24 a	44.46±0.41 a	43.05±0.38 b	43.40±0.39 a	43.17±0.31 a	42.76±0.40 a	48.94±0.16 b	47.14±0.16 a
1 mM	42.67±0.29 ab	44.70±0.62 a	43.50±0.11 a	44.22±0.45 a	42.54±0.11 a	40.99±0.21 bc	49.20±0.20 b	47.06±0.14 a
5 mM	42.58±0.25 ab	44.74±0.19 a	43.44±0.19 a	43.55±0.14 a	42.94±0.16 a	40.23±0.40 bc	49.71±0.20 a	47.25±0.39 a
10 mM	42.42±0.45 b	44.55±0.24 a	43.12±0.34 a	43.49±0.10 a	42.66±0.21 a	40.07±0.13 c	49.73±0.11 a	47.50±0.31 a
100 mM	42.45±0.13 b	43.59±0.08 a	44.43±0.15 a	44.13±0.64 a	43.17±0.20 a	41.05±0.14 b	48.92±0.03 b	47.39±0.59 a
x[c]	42.72±0.15**	44.41±0.18**	43.51±0.16**	43.76±0.18**	42.90±0.11**	41.02±0.28**	49.30±0.11**	47.27±0.14**
140 μmol · m^{-2} s^{-1} PAR (treatment ML)								
0 mM	44.87±0.46 a	44.15±0.21 b	42.63±0.12 c	44.77±0.53 ab	44.29±0.12 a	43.73±0.33 a	48.69±0.19 a	46.31±0.27 ab
1 mM	43.94±0.24 b	44.45±0.21 b	44.13±0.33 b	44.58±0.74 b	43.78±0.11 a	42.34±0.03 b	48.60±0.33 a	45.92±0.37 b
5 mM	44.26±0.18 ab	45.92±0.18 a	45.36±0.10 a	44.84±0.22 ab	44.12±0.06 a	41.88±0.33 b	49.04±0.08 a	46.87±0.41 ab
10 mM	44.97±0.15 a	46.12±0.09 a	45.66±0.16 a	45.65±0.09 ab	43.77±0.27 a	42.16±0.09 b	49.25±0.21 a	46.81±0.34 ab
100 mM	44.39±0.16 ab	44.56±0.07 b	45.79±0.26 a	46.04±0.21 a	44.01±0.13 a	42.63±0.20 b	48.95±0.09 a	47.33±0.21 a
x	44.49±0.14	45.04±0.23	44.71±0.34	45.18±0.22	43.99±0.08	42.55±0.19	48.91±0.10	46.65±0.18

[a] leaves # 10 and older from the shoot apex [b] leaves # 1 through 4 from the shoot apex
[c] Means between light regimes differ at ** P < 1 %

ganic N, irrespective of soil N level (Fig. 3: A). Besides traces of aspartic acid and serine, the sole organic N compounds detectable above the analysis threshold were glutamine (Gln) and, to a lesser extent, glutamic acid (Glu). No significant N effect on other xylem solutes could be noticed (Fig. 3: B). While the impact of light restriction on xylem P was minor, the Mg content was 2 to 3 times lower in LL compared to ML,the effect on K being intermediate.

In the vine, N concentrations were most elevated in sink leaves (lateral and young main leaves), followed in decreasing order by medium main leaves (not shown), source leaves (old main leaves), lateral shoots, inflorescences, main shoots and roots, and finally the trunk (Tab. 2). In either light regime, the N concentration in the inflorescences was below that in old leaves, which was only 72 % (ML) or even 59 % (LL), respectively, of that in young leaves. While there was no difference in N concentration in the fresh weight between light treatments (data not shown), light restriction enhanced the N concentration in the dry weight of all annual plant parts by 34 to 86 %, relative to ML, slightly (however significantly) reduced it in the trunk and had no effect on the roots. The proportion of N that was located in the plant canopy was higher in LL (61 %) than in ML (52.5 %), and the response to light restriction was more notable in sink leaves than in source leaves and inflorescences. High N fertilization rates increased N concentrations in all organs, and this increase was stronger at the higher irradiance. Yet, the N concentrations were equal at N5 and N10 and only slightly higher at N100. The most significant N effect was found in the main shoots and the least in the trunk.

The highest C concentrations in the dry weight were measured in the trunk, followed in decreasing order by the roots, sink leaves and inflorescences, medium main leaves (not shown), source leaves and main shoots, and finally lateral shoots (Tab. 3). Light limitation significantly reduced C contents in all annual organs of the vine and enhanced them in the perennial organs. The response to light restriction was more pronounced in source leaves and inflorescences than in sink leaves. There was only a minor influence of N supply, which was inconsistent and depended on the type and age of the tissue. This is best documented, if the different leaf age classes are compared. In the ML regime, the C concentration peaked at N100 in the lateral leaves, which were the youngest of all leaves, at N10 in the young main leaves, at N5 in the medium main leaves, and no N effect was found in the old main leaves. In LL however, high N levels decreased the C concentration in all but the lateral leaves. From Tab. 2 and 3 it can also be concluded that the C : N ratio decreased with increasing N supply in all plant parts, and this ratio was considerably higher in LL than in ML.

Non-structural carbohydrates in both wood and root samples were drastically depleted by light restriction, and the decrease in starch almost entirely accounted for this effect (Fig. 4). The responses to N supply were, however, different in the two light treatments. There was no consistent N effect in LL, while in ML the concentration of non-structural carbohydrates strongly declined as N supply increased. In the LL regime, soluble sugars correlated negatively with N concentration in the trunk ($r = -0.63$, $P < 5$ %), but not in the roots ($r = -0.42$, n.s.), while there was a positive relationship between starch and N in the

roots (r = 0.58, P < 5 %), but not in the trunk (r = -0.03, n.s.). In the ML treatment, however, the relationship between soluble sugars and N concentration was not significant in either the roots (r = -0.41, n.s) or the trunk (r = -0.36, n.s.), whereas starch correlated negatively with N in both the roots (r = -0.74, P < 5 %) and the trunk (r = -0.69, P < 5 %)The proportion of C in the non-structural carbohydrate fraction is indicated approximately by the ratio 0.4 · (starch + sugar) : C, since C accounts for 40 % of the molecular weight in glucose and 42 % in sucrose. It can be estimated from the data presented in Tab. 3 and Fig. 4 that this ratio was sharply reduced by low light and decreased with increasing N supply (in ML only) in both trunk and roots. In the trunk, 9 % (ML) or 5 % (LL) of the C was located in the non-structural carbohydrate fraction, whereas in the roots the proportion was 22 % (ML) or 13 % (LL), respectively.

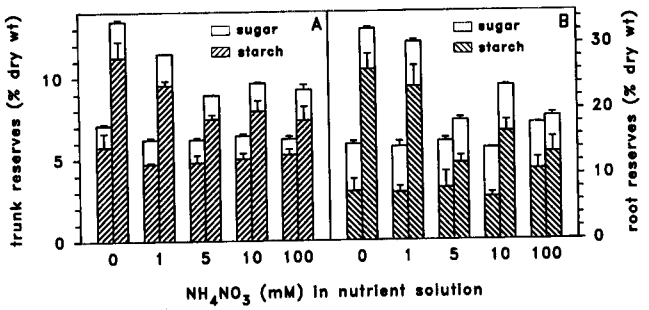

Fig. 4: Effect of irradiance and N application during bloom on non-structural carbohydrates in potted grapevines in the phytotron. Concentrations of starch and soluble sugars in the trunk (**A**) and in the roots (**B**). [Left and right bar of each pair: see Fig. 2]. Values are means ± SE (n = 3).

Discussion

The contents of NO_3^- and NH_4^+ in the soil and in the xylem sap indicate that NH_4^+ was rapidly nitrified in the soil, and NO_3^- was the primary mineral N compound taken up by the roots, although the two ions were applied at equal rates. However, the high soil pH may have effected some NH_3 volatilization by raising the NH_3 : NH_4^+ ratio. Nitrification processes, on the other hand, may have accounted for the slight decline in soil pH at elevated N supply rates and the subsequent changes in the concentrations of macro nutrients. The capacity of applied N to alter soil availability of other nutritional elements implies that massive N fertilization not only enhances the leaching potential of NO_3^- but also that of K, Ca and Mg and thus, in the long-term, increases the risk of various nutrient deficiencies. The results from our xylem solute analysis are in agreement with the statement of CHAPIN (1991) that nutrient uptake is curtailed under light-limiting conditions both by reduced root : shoot ratio and reduced potential of the roots to absorb nutrients, especially if nutrients are abundant. Low irradiance consequently accentuates leaching of ions, in particular Mg, which was the xylem compound most severely affected.

The relatively large concentrations of glutamine and glutamic acid in the xylem sap of the ML vines were not surprising, since inorganic N taken up by the roots is usually immediately metabolized to these organic transport forms (STOEV *et al.* 1966, ROUBELAKIS-ANGELAKIS and KLIEWER 1979, ANDERSEN and BRODBECK 1991, ALLEWELDT and MERKT 1992). The absence of additional organic compounds is in contradiction to the data obtained by the above mentioned authors, indicating that, by using our extraction technique, contaminations with phloem sap are omitted. However, the energy and carbohydrate requirements for NO_3^- reduction and NH_4^+ assimilation are high, and hence, the roots' capacity for N assimilation is limited and depends upon the translocation of sucrose from the shoots. Yet, starch synthesis has priority over sucrose synthesis in source leaves stressed by low irradiance (reviewed by GEIGER and SERVAITES 1991). The responses of C and N partitioning among the various plant parts to low light imply that C supply to the root was restricted. Thus, a considerable portion of the C required to sustain metabolic activities must have been derived from C substrates in the root, as indicated by the proportion of C in the non-structural fraction.

In the absence of photosynthates, the demand for reductant, energy and C skeletons placed by N assimilation may partially be met by stimulation of respiratory glycolysis (GEIGENBERGER and STITT 1991) and tricarboxylic acid (TCA) cycle activity (BLOOM *et al.* 1992). However, when photosynthesis is restricted, a larger fraction of root respiration is devoted to maintenance processes rather than ion uptake, assimilation and transport (AMTHOR 1989). Hence, the remobilization of protein reserves in the root tissue for the supply of C skeletons to the TCA cycle, as proposed by ROBINSON *et al.* (1992), may have accounted for part of the NH_4^+ in the xylem sap. In accordance with PATE (1980), the ratio of organic N to NO_3^- in the xylem sap indicates that the roots' potential to assimilate N was exceeded already at intermediate N supply in ML and was close to zero in LL. Consequently, inorganic N was transported with the transpiration stream to the green plant parts. Nitrate may transiently be stored in petioles, but once it is imported into the leaves, it must be assimilated or stored permanently, since there is practically no re-export of the phloem-immobile NO_3^- from the leaf (JESCHKE *et al.* 1991). The contribution of the shoot to whole-plant N assimilation therefore increases as N uptake increases, particularly under low irradiance. In this context it is interesting to note that the vine's capacity for N assimilation appears to be higher in leaves than in roots (PÉREZ and KLIEWER 1978, ROUBELAKIS-ANGELAKIS and KLIEWER 1983).

Yet, there is also a close relationship between irradiance and NO_3^- reduction in leaves. DE CIRES *et al.* (1993) stated that C assimilation is the main factor controlling nitrate reductase (NR) activity in leaves rather than light *per se*. We have previously shown that the leaves' photosynthetic potential was limited by low irradiance in this experiment (KELLER and KOBLET1994). Those data together with the present results on C and N concentrations suggest that nutrient uptake is strikingly less affected by the light

environment than CO_2 and N assimilation, as described by ASLAM and HUFFAKER (1984) and RUFTY *et al.* (1989). Inorganic N therefore tends to accumulate in all plant parts at high N nutrition, in particular under limiting light conditions (MENGEL 1985). LÖHNERTZ (1988) could detect NO_3^- in all organs of grapevines during the whole season, and NO_3^- concentrations were influenced by soil management techniques, which affected N availability. PÉREZ and KLIEWER (1982) found NO_3^- concentrations in grape petioles to be directly related to N nutrition, when the vines were grown under reduced irradiance but not in full sunlight. They also reported a positive relationship between NR activity and irradiance and an inverse relationship between NR activity and NO_3^- accumulation in the leaves. Similar results for NR activity and NO_3^- as well as NH_4^+ accumulation in leaf blades and petioles were obtained by SMART *et al.* (1988) and PÉREZ HARVEY and VALDÉS LAURSEN (1989) with grapevines grown under different shade levels.

Although soil and xylem sap analyses both indicated reduced rates of N uptake under light stress, there was no effect on the amounts of total N per vine (data not shown). In addition, increased N concentrations in the annual organs of LL vines are in contradiction to the general findings that tissue N contents decline in light-limited plants (EVANS 1989). Yet, GULMON and CHU (1981) reported increased N concentrations per unit leaf weight, and SCHULZE *et al.* (1991) also found elevated N concentrations in plants grown under light restriction. First, these results reveal a problem with fertilization recommendations based on leaf analysis: if leaves are sampled after a period of overcast conditions, even N deficient plants may appear well supplied, and thus, no fertilization would be recommended. Second, increases in N concentrations and simultaneous decreases in C concentrations in response to low-light stress are only possible, if a considerable portion of this N is inorganic. And third, equal amounts of N per vine in both light regimes can only be explained, if one assumes some kind of N loss, which must have been larger in ML than in LL.

The light level in LL was around the light compensation point of photosynthesis, and hence, net C gain achieved by these plants during imposition of the light stress was negligible (KELLER and KOBLET 1994). Nevertheless, active growth continued in either light regime during the course of the experiment, and the amount of total N in the new growth exceeded the potential supply from the soil in N0 and N1. This must be related to recirculation of C and N within the vine by sequential senescence of old leaves (WERMELINGER and KOBLET 1990) and withdrawal of reserves from trunk and roots, since maximal C gain occurs if N is partitioned to the best exposed leaves to stimulate photosynthesis (MOONEY and GULMON 1979). Remoblized N may account for > 60 % of the total N in the youngest leaf blades (MAE *et al.* 1985), and NH_3 is released by the hydrolysis of Rubisco (the principal source of transported N), other proteins and chlorophyll in older tissues. RABE (1990) assumed that any stress causing glucose depletion and/or reduced growth results in NH_3 accumulation, and FARQUHAR *et al.* (1978) reported emission of NH_3 from senescing maize leaves, which were still photosynthetically active. We have hypothesized that the larger stomatal conductance in the ML plants may have permitted NH_3 evolution preventing a toxic accumulation of NH_3 in leaves and inflorescences (KELLER and KOBLET 1994). On the other hand, even in ML the severity of inflorescence necrosis, described in the same article, did not reflect the strong increase in N concentration in the flower clusters with increasing N supply.

The levels of non-structural carbohydrates in the permanent organs were identical in all vines before the imposition of the stress treatments. However, the decrease in starch content with increasing N supply in both the trunk and roots in ML cannot fully be attributed to an enhanced growth response. Although the lowest concentrations were found in the N5 vines showing themost active shoot growth, the N100 plants also had very low starch levels. Yet, net growth of these plants was reduced to the level of the N0 plants (data not shown). By contrast, photosynthetic CO_2 fixation and C contents and, therewith, net C acquisition remained fairly constant over the entire range of N levels. This discrepancy is also expressed in the proportion of C in the non-structural carbohydrate fraction. Inorganic N assimilation diverts C from the synthesis of sucrose and starch toward the synthesis of amino acids (VAN QUY *et al.* 1991, CHAMPIGNY and FOYER 1992) and organic acids (ROBINSON and BAYSDORFER 1985). Therefore, there is a close and inverse correlation between N nutrition and carbohydrate accumulation (discussed in MAKINO and OSMOND 1991, CHAPIN 1991). Low light levels and high N supply, hence, independently decrease the amount of sucrose available for export to the various sink organs. Elevated rates of N uptake and assimilation may thus have depleted the C reserves and/or inhibited replenishment of the reserves used for early growth. Although N had been applied 11 d before imposition of the light regimes, non-structural carbohydrates in the LL vines failed to respond to N supply. This suggests that a certain fraction of C was located in storage areas and unavailable for metabolism (RUFTY *et al.* 1989).

We concluded that the main leaves are clearly not the only source organs during bloom, and remobilization of reserves from the permanent parts may be continued or reinitiated beyond bloom, at least under stress conditions, to sustain essential growth and maintenance processes in order to guarantee survival of the vine. The present results provide further evidence that flower clusters are low-priority sinks compared to shoot tips and support our hypothesis that C starvation rather than excessive N uptake, is the cause of this so-called disorder.

Acknowledgements

We are grateful to Dr. M. CARMO CANDOLFI-VASCONCELOS, Department of Horticulture, Oregon State University, Corvallis,

Oregon USA, and P. PERRET for their helpful advice and critical reading of the manuscript. We thank ANNA DÜRSTELER for her assistance in tissue analysis. This research was part of a Ph.D. thesis of M. KELLER supervised by Prof. Dr. J. NÖSBERGER, Swiss Federal Institute of Technology, Department of Plant Science, CH-8092 Zürich, Switzerland. Financial support was provided by the Swiss Viticulture Fund (Schweizerischer Weinbaufonds).

Literature cited

ALLEWELDT, G.; MERKT, N.; 1992: Der Stickstoffexport der Wurzel und die Zusammensetzung des Xylemexsudats. Teil 1: Der Einfluss einer zunehmenden Stickstoffdüngung. Vitis **31**, 121-130.

AMTHOR, J. S.; 1989: Respiration and Crop Productivity. Springer-Verlag, New York.

ANDERSEN, P. C.; BRODBECK, B. V.; 1991: Influence of fertilization on xylem fluid chemistry of *Vitis rotundifolia* Noble and *Vitis* hybrid Suwannee. Amer. J. Enol. Viticult. **42**, 245-251.

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.

BLOOM, A. J.; SUKRAPANNA, S. S.; WARNER, R. L.; 1992: Root respiration associated with ammonium and nitrate absorption and assimilation by barley. Plant Physiol. **99**, 1294-1301.

CANDOLFI-VASCONCELOS, M. C.; KOBLET, W.; 1990: Yield, fruit quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis vinifera*. Evidence of compensation and stress recovering. Vitis **29**, 199-221.

CHAMPIGNY, M. L.; FOYER, C.; 1992: Nitrate activation of cytosolic protein kinase diverts photosynthetic carbon from sucrose to amino acid biosynthesis. Plant Physiol. **100**, 7-12.

CHAPIN, F. S., III; 1991: Integrated response of plants to stress. A centralized system of physiological responses. BioScience **41**, 29-36.

CONRADIE, W. J.; 1991: Translocation and storage of nitrogen by grapevines as affected by time of application. In: RANTZ, J. M. (Ed.): Proc. Intern. Symp. Nitrogen Grapes Wine, Seattle Washington, USA. June 1991, 32-42. Cal Central Press, Sacramento.

DE CIRES, A.; DE LA TORRE, A.; DELGADO, B.; LARA, C.; 1993: Role of light and CO_2 fixation in the control of nitrate-reductase activity in barley leaves. Planta **190**, 277-283.

EVANS, J. R.; 1989: Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia **78**, 9-19.

FARQUHAR, G. D.; WETSELAAR, R.; FIRTH, P. M.; 1978: Ammonia volatilization from senescing leaves of maize. Science **203**, 1257-1258.

GEIGENBERGER, P.; STITT, M.; 1991: Regulation of carbon partitioning between sucrose and nitrogen assimilationin cotyledons of germinating *Ricinus communis* L. seedlings. Planta **185**, 563-568.

GEIGER, D. R.; SERVAITES, J. C.; 1991: Carbon allocation and responses to stress. In: MOONEY, H. A.; WINNER, W. E.; PELL, E. J. (Eds.): Response of Plants to Multiple Stresses, 103-127. Academic Press, San Diego.

GOLDSPINK, B.; GORDON, C.; 1991: Response of *Vitis vinifera* cv. Sauvignon blanc grapevines to timed applications of nitrogen fertilizer. In: RANTZ, J. M. (Ed.): Proc. Intern. Symp. Nitrogen Grapes Wine, Seattle Washington, USA. June 1991, 255-258. Cal Central Press, Sacramento.

GULMON, S. L.; CHU, C. C.; 1981: The effects of light and nitrogen on photosynthesis, leaf characteristics, and dry matter allocation in the chaparral shrub, *Diplacus aurantiacus*. Oecologia **49**, 207-212.

HO, L. C.; 1988: Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Ann. Rev. Plant Physiol. Plant Mol. Biol. **39**, 355-378.

JESCHKE, W. D.; WOLF, O.; PATE, J. S.; 1991: Solute exchanges from xylem to phloem in the leaf and from phloem to xylem in the root. In: BONNEMAIN, J. L.; DELROT, S.; LUCAS, W. J.; DAINTY, J. (Eds.): Recent Advances in Phloem Transport and Assimilate Compartmentation, 96-105. Ouest Editions, Presses Académiques, Nantes.

KELLER, M.; KOBLET, W.; 1994: Is carbon starvation rather than excessive nitrogen supply the cause of inflorescence necrosis in *Vitis vinifera* L.? Vitis **33**, 81-86.

KOBLET, W.; 1969: Wanderungen von Assimilaten in Rebtrieben und Einfluss der Blattfläche auf Ertrag und Qualität der Trauben. Wein-Wiss. **24**, 277-319.

KÜNSCH, U.; SCHÄRER, H.; TEMPERLI, A.; 1977: Zur Automatisierung der Bodenanalyse. Schweiz. Landw. Forsch. **16**, 141-153.

LÖHNERTZ, O.; 1988: Untersuchungen zum zeitlichen Verlauf der Nährstoffaufnahme bei *Vitis vinifera* (cv. Riesling). Diss. Justus-Liebig-Univ. Giessen. Geisenheimer Ber. **1**, BR Deutschland.

MAE, T.; HOSHINO, T.; OHIRA, K.; 1985: Protease activities and loss of nitrogen in the senescing leaves of field-grown rice (*Oryza sativa* L.). Soil Sci. Plant Nutr. **31**, 589-600.

MAKINO, A.; OSMOND, B.; 1991: Effects of nitrogen nutrition on partitioning between chloroplasts and mitochondria in pea and wheat. Plant Physiol. **96**, 355-362.

MENGEL, K.; 1985: Aufnahme und Reduktion von Nitrat sowie Nitratgehalte in Pflanzen. Landw. Forsch., Kongressband 96. VDLUFA-Kongress, Karlsruhe. September 1984, 146-157. Sauerländer Verlag, Frankfurt am Main.

MOONEY, H. A.; GULMON, S. L.; 1979: Environmental and evolutionary constraints on the photosynthetic characteristics of higher plants. In: SOLBRIG, O. T.; JAIN, S.; JOHNSON, G. B.; RAVEN, P. H. (Eds.): Topics in Plant Population Biology, 316-337. Columbia Univ. Press, New York.

PATE, J. S.; 1980: Transport and partitioning of nitrogenous solutes. Ann. Rev. Plant Physiol. **31**, 313-340.

PEACOCK, W. L.; CHRISTENSEN, L. P.; BROADBENT, F. E.; 1989: Uptake, storage, and utilization of soil-applied nitrogen by Thompson Seedless as affected by time of application. Amer. J. Enol. Viticult. **40**, 16-20.

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.

PÉREZ HARVEY, J.; VALDÉS LAURSEN, M.; 1989: Efecto de la fertilización nitrogenada y el sombreamiento sobre la fructificación y necrosis de yemas en el cv. Sultanina (Thompson Seedless). Cienc. Inv. Agr. **16**, 89-98.

PERRET, P.; 1993: Begrünung im Weinbau: Optimierung des zeitlichen Stickstoffangebotes durch Bodenpflegemassnahmen. Dt. Weinbau-Jahrbuch **44**, 81-88.

RABE, E.; 1990; Stress physiology: The functional significance of the accumulation of nitrogen-containing compounds. J. Hort. Sci. **65**, 231-243.

ROBINSON, J. M.; BAYSDORFER, C.; 1985: Interrelationships between carbon and nitrogen metabolism in mature soybean leaves and isolated leaf mesophyll cells. In: HEATH, R. L.; PREISS, J. (Eds.): Regulation of Carbon Partitioning in Photosynthetic Tissues, 333-357. Amer. Soc. Plant Physiologists, Rockville, MD, USA.

ROBINSON, S. A.; STEWART, G. R.; PHILLIPS, R.; 1992: Regulation of glutamate dehydrogenase activity in relation to carbon limitation and protein catabolism in carrot cell suspension cultures. Plant Physiol. **98**, 1190-1195.

ROUBELAKIS-ANGELAKIS, K. A.; KLIEWER, W. M.; 1979: The composition of bleeding sap from Thompson Seedless grapevines as affected by nitrogen fertilization. Amer. J. Enol. Viticult. **30**, 14-18.

- -; - -; 1983: Ammonia assimilation in *Vitis vinifera* L.: II. Leaf and root glutamine synthetase. Vitis **22**, 299-305.

RUFTY, T. W.; MACKOWN, C. T.; VOLK, R. J.; 1989: Effects of altered carbohydrate availability on whole-plant assimilation of $^{15}NO_3^-$. Plant Physiol. **89**, 457-463.

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

SCHULZE, W.; STITT, M.; SCHULZE, E. D.; NEUHAUS, H. E.; FICHTNER, K.; 1991: A quantification of the significance of assimilatory starch for growth of *Arabidopsis thaliana* L. Heynh. Plant Physiol. **95**, 890-895.

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.

STOEV, K. D.; DOBREVA, S. I.; WOSTENINEZ, G.; 1966: Über die Synthese von Aminosäuren im Wurzelsystem der Rebe. Vitis **5**, 265-287.

VAN QUY, L.; LAMAZE, T.; CHAMPIGNY, M. L.; 1991: Short-term effects of nitrate on sucrose synthesis in wheat leaves. Planta **185**, 53-57.

WERMELINGER, B.; KOBLET, W.; 1990: Seasonal growth and nitrogen distribution in grapevine leaves, shoots and grapes. Vitis **29**, 15-26.

WILLIAMS, L. E.; 1991: Vine nitrogen requirements - Utilization of N sources from soils, fertilizers, and reserves. In: RANTZ, J. M. (Ed.): Proc. Intern. Symp. Nitrogen Grapes Wine, Seattle Washington, USA. June 1991, 62-66. Cal Central Press, Sacramento.

WINKLER, A. J.; COOK, J. A.; KLIEWER, W. M.; LIDER, L. A.; 1974: General Viticulture. Univ. of California Press, Berkeley, Los Angeles.

YANG, Y. S.; HORI, Y.; 1979: Studies on retranslocation of accumulated assimilates in 'Delaware' grapevines. I. Retranslocation of ^{14}C-assimilates in the following spring after ^{14}C feeding in summer and autumn. Tohoku J. Agricult. Res. **30**, 43-56.

- -; - -; OGATA, R.; 1980: Studies on retranslocation of accumulated assimilates in 'Delaware' grapevines. II. Retranslocation of assimilates accumulated during the previous growing season. Tohoku J. Agricult. Res. **31**, 109-119.

Received October 10, 1994