

Starch Concentrations in Grapevine Leaves, Berries and Roots and the Effect of Canopy Management

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Diurnal and seasonal starch changes in leaves, berries and roots of *Vitis vinifera* L. cv. Cabernet Sauvignon/99 Richter and the effect of canopy management (a combination of suckering, shoot positioning, and 33% defoliation) on these processes were investigated under field conditions. An increase in starch concentration of basal leaves occurred from the morning to the afternoon during the most active vegetative growth period (up to véraison), indicating a proportional change in storage or export of assimilates between day and night. During later developmental stages diurnal starch levels slightly declined or remained the same. Seasonally, leaf starch remained relatively stable until véraison, whereafter it increased, reaching highest concentrations at the post-harvest stage. Canopy management generally increased leaf starch concentrations. Berries contained no significant amounts of starch. Root starch concentrations were usually higher than those of leaves. Diurnally as well as seasonally, root starch accumulation patterns coincided with those of leaves, indicating a close relationship between source and sink tissue. Canopy management resulted in stable diurnal root starch concentrations without affecting the daily mean starch level; afternoon values of treated vines were, however, generally lower. The results imply that carbohydrate supply and starch-synthesising enzyme systems were not limited by manipulating the canopy and decreasing foliage. It seems, however, that particularly late in the season starch accumulation in roots may be delayed by canopy manipulation as applied. This indicates a shift in sucrose partitioning and its utilisation in roots and other sink areas, e.g. the berries, when the canopy is manipulated.

Starch is known to be the main reserve compound in grapevine storage tissue (Winkler & Williams, 1945). In order to establish the effect of potentially stress-inflicting treatments on the longevity of grapevines, starch levels in perennial tissues (canes, trunk, roots) were repeatedly determined. Experiments included different levels of defoliation (Scholefield, Neales & May, 1978; Candolfi-Vasconcelos & Koblet, 1990; Hunter *et al.*, 1995), cropping (Buttrose, 1968; Balasubrahmanyam, Eifert & Diofasi, 1978), crop removal (Wample & Bary, 1992), nitrogen fertilisation (Korkas *et al.*, 1994), soil water supply (Hofäcker, 1977) and growth retardant (Hunter & Proctor, 1994).

Assimilates in higher plants are partitioned into cytoplasmic sucrose synthesis/export or transitory starch accumulation in the chloroplasts during the day (Upmeyer & Koller, 1973; Acock, Acock & Pasternak, 1990). Controversy exists about the relationship between photosynthate build-up in source leaves and the rate of carbon fixation (Neales & Incoll, 1968; Wareing, Khalifa & Treharne, 1968; Geiger, 1976; Goldschmidt & Huber, 1992). Sucrose accumulation is generally believed to be involved in an indirect inhibitory process. Support for this hypothesis was found in grapevines, particularly during the latter part of the growth season (Hunter, Skrivan & Ruffner, 1994). However, the nature of the control mechanism remains to be elucidated. Starch accumulation in the chloroplasts was originally also considered to be associated with a reduction in photosynthetic activity (Upmeyer & Koller, 1973; Thorne & Koller, 1974; Nafziger & Koller, 1976; Chatterton & Silvius, 1979; Herold, 1980), but this relationship was seriously questioned by Goldschmidt & Huber (1992). Nevertheless, the concept that the extent of accumula-

tion and location of carbohydrates in the grapevine canopy may affect photosynthetic performance, with implications for growth and development, yield and grape composition, is still valid. Occasional determination of starch in source leaves (Buttrose & Hale, 1971; Downton & Hawker, 1973; Hofäcker, 1977; Chaumont, Morot-Gaudry & Foyer, 1994), does not change the fact that field-grown vines have not been systematically investigated in this respect. Pertinent knowledge will contribute to our understanding of the regulatory processes taking place in the grapevine and is needed to optimise canopy management strategies which guarantee sustainable high-quality yields.

In previous studies we showed that the photosynthetic activity in the leaves increases when source size is reduced by canopy management practices, e.g. partial defoliation (Hunter & Visser, 1988a, 1988b; Hunter *et al.*, 1995). Improved canopy microclimate, exerting a stimulating effect on both source and sink metabolic activity, as well as increased sink:source ratios were offered as the most likely explanations. Higher levels of carboxylating enzymes and growth hormones were also suggested (Wareing *et al.*, 1968). Nevertheless, from our and many other studies in which the leaf area of plants was reduced, it became evident that plants normally function at photosynthetic levels well below their maximum capacity. Manipulating the canopy and leaf environment, as is commonly done in viticulture, must therefore affect photosynthate partitioning more than assimilate availability. Since basal leaves just above the bunches were identified as being primarily responsible for supporting the bunches throughout the growth season (Hunter & Visser, 1988a, 1988c), it seemed reasonable to monitor the accumulation of carbohydrates in these

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leaves.

The aim of this investigation was to establish normal diurnal and seasonal starch accumulation patterns in grapevine leaves, berries and roots and to determine the effect of canopy management on these processes. The results are discussed with reference to diurnal as well as seasonal photosynthetic activities and sucrose levels reported previously (Hunter *et al.*, 1994).

MATERIALS AND METHODS

Experimental vineyard: Eleven year old Cabernet Sauvignon (clone CS 46) vines, grafted onto 99 Richter (clone RY 30), were studied in the Western Cape at Nietvoorbij, Stellenbosch. Vines were grown with 3.0 x 1.5 m spacing on a Glenrosa soil (Series 13, Kanonkop) (MacVicar *et al.*, 1977), trained to a 1.5 m slanting trellis (described by Zeeman, 1981) and pruned to 10 buds per kg cane mass. Vines received 50 mm supplementary irrigation just after the berries reached pea size and at véraison.

Treatments: Two treatments, namely control and canopy management, were applied. The latter comprised a combination of removing at 30 cm length all shoots not situated on two-bud spurs (suckering), positioning shoots in line with spurs, removal of every third leaf in the zone opposite and below bunches at berry set, and removal of 33% of the leaves in the remainder of the lower half of the canopy at pea size stage. One per every three leaves was removed, starting at the basal end of the shoot.

Sampling: Leaves: The first three basal leaves above the upper bunch were sampled. Berries: The main bunches on the shoot were harvested, destemmed and a representative sample of the berries used for further analysis. Roots: A representative root sample consisting of all root sizes was obtained by boring holes of approximately 7 cm in diameter and 30 cm depth randomly at 30 cm distance from the vine trunk. Roots were retrieved from the soil by careful washing.

Sampling took place at 10:30 and 15:30 at berry set, pea size, véraison, ripeness and post-harvest (one month after harvest) stages. Leaves and bunches were sampled from one shoot on each of four vines, whereas roots were sampled from three vines, at each sampling time. A composite root sample was used for analysis at each sampling time. Leaves, berries and roots were processed immediately.

Starch determination: Soluble sugars were extracted from fresh leaf, berry and root tissue with MeOH-CHCl₃-0.2M HCO₂H (12:5:3 v/v) as described by Hunter *et al.* (1995). The dry residue was used for starch analysis: A 50 mg sample was weighed into an Eppendorf tube, 1 cm³ 80% aqueous acetone added, and the suspension vortexed for 10 sec and sonicated for 10

min. The suspension was left at 4°C for 6h, centrifuged (Eppendorf), and the supernatant decanted. One cm³ ethanol was then added to the residue. Except for the time lapse between sonication and centrifugation, the above procedure was repeated. After addition of 1 cm³ water, the residue was washed again, frozen at -20°C, freeze-dried overnight, 550 mm³ water added, vortexed (10 sec) and sonicated (10 min). The sample was then left at 4°C for 60 min and centrifuged (10 min). A 50 mm³ aliquot was removed as control.

The sample was heated in a boiling water bath (5 min with open caps and 55 min closed) to induce gelatinisation of starch. After cooling, 500 mm³ of an enzyme mix containing 5 U α -amylase (Sigma A-6380) and 2 U amyloglucosidase (Sigma A-7255) in 0.1 M Na-acetate (acetic acid/Na-acetate) buffer (pH 5.0) was added, the mixture vortexed for 10 sec and incubated at 40°C under constant shaking (35 r.p.m.) to hydrolyse starch (vials were vortexed for 10 sec every 30 min). After 3h the samples were centrifuged (10 min) and diluted (1:39) with water. Controls were not incubated.

Generation of glucose from starch was determined by using the ABTS [2,2' azino-di (3 ethylbenzthiazoline)-6'-sulfonate] reagent, containing 3.45 g Na₂HPO₄·2H₂O, 1.6 g NaH₂PO₄·H₂O, 2350 U glucose oxidase (Boehringer no. 646423), 375 U peroxidase (Boehringer no. 127361) and 125 mg ABTS (Boehringer no. 102946), in 250 cm³ water.

An aliquot (50 mm³) of the diluted sample was mixed with 950 mm³ of the reagent. Absorbance was read at 436 nm after 30 min. The blank consisted of reagent and water. To obtain a glucose standard curve, seven standards of 0, 5, 10, 20, 30, 40 and 50 mg glucose/100 cm³, were prepared. Results are expressed in mg starch after multiplication with a factor of 0.9, which allows for the reduced molecular weight of glucose in the polymer. Control values are subtracted.

Experimental design and statistical analyses: The experiment was laid out as a completely randomised design. Treatments were applied for two consecutive years. No interaction was found between years, and starch concentrations did not differ. A one-way analysis of variance was performed on the raw data. Student's t-test was used to test for significant differences between treatment means.

RESULTS AND DISCUSSION

Leaves: In general, starch accumulation in basal leaves tended to increase from the morning to the afternoon during the first part of the growth season (up to véraison), particularly in untreated vines (Table 1). This pattern coincides with the time-course of sucrose synthesis found under normal field conditions, as well as after extended predarkening of vines (Hunter *et al.*, 1994). It would seem to reflect the expected daily accumulation

observed in plants grown under conditions favouring high CO₂ fixation rates (Upmeyer & Koller, 1973; Chatterton & Silviu, 1979; Davis & Loescher, 1991; Ghiena, Schultz & Schnabl, 1993). In a preliminary time-course study carried out at different developmental stages of the vine, almost 20% lower starch concentrations were observed in apical than in basal leaves (data not shown). Although the diurnal pattern diverges, higher photosynthetic rates were found for recently matured leaves during these stages (Hunter & Visser, 1988a, 1988c; Hunter *et al.*, 1994), which satisfy the carbon demand of nearby growing sinks (Ruffner, Adler & Rast, 1990; Hunter *et al.*, 1994).

The data indicate that, in line with the situation prevailing in most starch-storing plant species, a portion of newly fixed carbon is retained in the laminae during the day in polymerised form and exported at night. In view of the number of sinks active in perennial plants such as the grapevine early in the season, the preference for transitory starch accumulation instead of sucrose export during the day may be considered the result of a limited transport capacity and indicates that starch synthesis proceeds independently of sink demand for sucrose.

At ripeness and post-harvest stages starch concentrations of basal leaves were already high in the morning and little change occurred during the day, indicating that the transitory pools were more or less constantly filled as a result of slow nocturnal assimilate export. Simultaneous sharp increases in sucrose concentrations, diurnally as well as seasonally, were reported previously for this period, whereas photosynthetic rates already started to decrease at the onset of ripening (Hunter *et al.*, 1994). The observed increase in leaf starch levels at ripeness marks the start of annual reserve accumulation,

increasing sharply towards the post-harvest stage. After the cessation of vegetative growth (Hunter & Visser, 1990), both supply of and demand for photosynthates decrease. The persisting CO₂ assimilation by basal leaves after harvest (Hunter *et al.*, 1994) is able to sustain carbohydrate supply while demand is dwindling. This leads to an increase in the supply: demand ratio and contributes to an accumulation of untranslocated starch in the source tissue. Starch levels were found to display an inverse relationship to photosynthesis on a diurnal as well as on a seasonal basis. The available data on carbohydrate concentrations and photosynthetic activities of young and mature leaves clearly indicate the responsiveness of assimilation to the requirements of sinks.

Canopy management improves light conditions and allows higher photosynthetic rates while at the same time sink:source ratios are changed (Hunter *et al.*, 1995). As a result, foliar starch concentrations increased in the morning. The pattern of accumulation was similar to that in untreated vines. The response to light is similar to that found by Chatterton & Silviu (1981) for soybean leaves grown under controlled conditions. The lower starch content of control leaves in the morning implies that they were either depleted to a greater extent than treated leaves during the night or transitory starch pools were filled at a faster rate in treated leaves in the early morning. It is also possible that foliage removal reduced carbon drain, conceivably by slowing down export and/or decimation of local sinks including dark respiration of leaves still on the vine. However, this argument is incongruous with, e.g. the higher yields found with partial defoliation alone (Hunter *et al.*, 1995). It is evident that although starch may be involved in the regulation of photosynthesis, mechanisms with a bearing on starch formation must also come into play when the grapevine

TABLE 1

Morning (10.30) and afternoon (15.30) starch content in grapevine leaves determined at different developmental stages.

Developmental stage	Morning values (mg.g ⁻¹ dry mass)		Afternoon values (mg.g ⁻¹ dry mass)		*Diurnal accumulation factor		Daily mean	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Berry set	48,1d	89,8bc	70,2c	85,1bc	1,5	1,0	59,1f	87,4cde
Pea size	48,5d	60,8cd	99,0abc	62,1c	2,0	1,0	73,7def	61,5ef
Véraison	64,3cd	66,8cd	69,9c	79,4c	1,1	1,2	67,1ef	73,1def
Ripeness	109,8ab	109,2b	98,3abc	89,6abc	0,9	0,8	104,0bc	99,4bcd
Post-harvest	124,9ab	145,5a	124,3ab	130,7a	1,0	0,9	124,6ab	138,1a

Values designated by the same letter within each main column do not differ significantly ($p \leq 0.05$).

*Afternoon value divided by morning value.

grapevine canopy is altered by canopy management through, e.g. foliage removal. An involvement of endogenous plant growth regulators, e.g. abscisic acid, gibberellin, cytokinins and auxin, may be an explanation (Sweet & Wareing, 1966; Wareing *et al.*, 1968; Weaver, Shindy & Kliwer, 1969; Kliwer & Fuller, 1973; Geiger, 1976, and references therein). In this context it seems noteworthy that partial defoliation was consistently found to stimulate growth of secondary (fine and extension) grapevine roots (Hunter & Le Roux, 1992; Hunter *et al.*, 1995).

Berries: Despite the abundance of starch precursors like glucose, fructose and sucrose, only traces of starch were detectable in berries (data not shown).

Roots: In control vines, root starch concentrations generally increased during the day (Table 2). However, when canopy management was applied diurnal levels remained virtually constant. Afternoon values were normally lower in roots of treated than of untreated plants, whereas in the morning the respective levels were generally higher or the same. Apparently, under canopy management conditions excess carbohydrate formed during the day was redirected to supply in the requirements of other sinks, e.g. the fruits. The lower starch levels, particularly at ripeness and post harvest stages, after foliage reduction may also indicate an impairment of starch accumulation. Although it stands to reason that stored starch can be mobilised from perennial parts of the plant under severe stress when shoots are completely defoliated or defoliated to just a few leaves (Candolfi-Vasconcelos, Candolfi & Koblet, 1994, and references therein), it is doubted whether critically low levels of

starch were reached in this study. However, it is clear that not only photosynthesis but also carbon partitioning were affected by canopy management. In view of available data (Hunter *et al.*, 1995), it is conceivable that a redirection of carbon occurred, which is beneficial to the vine and the grape.

The low starch content in roots of grapevines (Yang, Hori & Ogata, 1980) and apple trees (Hansen, 1977) reflects the high carbohydrate demand of vegetative growth in spring. The general built-up of starch from berry set to the post harvest stage coincides with the pattern observed in leaves. This indicates that carbohydrate availability increases during the vegetation period and carbon partitioning between leaves and roots is interrelated. Close relationships between above-ground and subterranean growth of grapevines are known to exist (Hunter *et al.*, 1995, and references therein).

Root starch concentrations after berry set matched or were higher than those in leaves, but even highest post-harvest values were lower than those determined previously in different classes of roots during dormancy (Hunter *et al.*, 1995). It is assumed that the leaves were photosynthetically active and continued to supply perennial storage tissue with carbohydrate even after the last sampling stage. This confirms the findings of Scholefield *et al.* (1978). It is also possible that starch recycling and carbon as well as nitrogen export from leaves just prior to abscission may boost the carbohydrate reserve pool. The patterns of accumulation and remobilisation of root starch correspond closely to the general annual cycle of carbon metabolism found in other perennials (Loescher, McCamant & Keller, 1990).

TABLE 2

Morning (10.30) and afternoon (15.30) starch content in basal grapevine roots determined at different developmental stages.

Developmental stage	Morning values (mg.g ⁻¹ dry mass)		Afternoon values (mg.g ⁻¹ dry mass)		*Diurnal accumulation factor		Daily mean	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Berry set	10,2	31,6	48,8	31,6	4,8	1,0	29,5c	31,6c
Pea size	88,0	94,0	97,1	89,5	1,1	1,0	92,5bc	91,8bc
Véraison	124,7	118,7	103,1	113,2	0,8	1,0	113,9ab	115,9ab
Ripeness	86,0	74,4	151,9	84,5	1,8	1,1	119,0ab	79,5bc
Post-harvest	146,4	190,7	238,0	180,6	1,6	1,0	192,2a	185,6a

Values designated by the same letter do not differ significantly ($p \leq 0.05$).

The sampling method prevented normal statistical analyses on the morning and afternoon values.

*Afternoon value divided by morning value.

The observed fluctuations also follow the seasonal changes in dry mass (Conradie, 1980).

CONCLUSIONS

Diurnal starch accumulation in grapevine basal (source) leaves before the onset of ripening, i.e. during the phase of most active vegetative growth, was found to be largely independent of diligent canopy management (shoot and leaf removal). Although starch contents in roots were normally higher than in leaves, the situation observed above ground was largely paralleled. However, in both leaves and roots, differences in starch remobilisation and carbon partitioning occurred between treated and control plants, resulting in lower pre-véraison morning carbohydrate levels in leaves, and generally higher afternoon levels in the roots of control plants. The results indicate that a more complex mechanism than a simple feedback inhibition by starch and/or sucrose is involved in the regulation of photosynthetic activity, at least in grapevines grown under field conditions which have to partition carbon between photosynthesising, reproductive and perennial storage tissue. Canopy manipulation obviously had an effect on the direction of translocation and accumulation of carbohydrate. In line with previous observations, it seems that canopy management as applied succeeded in redirecting carbohydrate to the benefit of other sinks.

Basal leaves continued to supply carbohydrate to perennial storage tissue after harvest. Injudicious and severe defoliation of basal leaves at any time during the growth season will therefore harm reserve accumulation, which may have serious implications for growth and development of vegetative as well as reproductive tissue in the following season. This may have a particular effect under potentially stressful conditions, e.g. winter frost, dry summer periods, overcropping and excessive nitrogen fertilisation forcing fast canopy development.

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