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Diurnal and seasonal physiological changes in leaves of *Vitis vinifera* L.: CO_2 assimilation rates, sugar levels and sucrolytic enzyme activity

by

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S u m m a r y : Changes in photosynthetic rates, sugar contents, and sucrolytic activity (invertase and sucrose synthase) in young (apical) and mature (basal) leaves of *Vitis vinifera* L. were investigated throughout the growth season. Photosynthetic activity of basal leaves was predominant before the berries reached pea size, but declined to low rates during grape ripening and after harvest. Apical leaves, on the other hand, showed a more or less uniform pattern of photosynthesis during the whole season with higher assimilation rates than basal leaves after the onset of ripening (veraison). High photosynthetic rates in young material are likely to be sustained by a continuous demand for assimilates from local or adjacent sinks.

Diurnal fluctuations of sucrose, glucose, and fructose were similar in apical and basal leaves. Other than the photosynthetic rates, sucrose levels in young leaves tended to be somewhat higher than in mature leaves early in the season, but this balance was reversed after veraison. Generally, low sucrose concentrations correlated with high hexose values (berry set) and *vice versa* (post-harvest). Increased sucrose levels were observed under conditions where carbon import or export limitation is expected to prevail.

In comparison with the presumably normal situation observed before veraison, apical leaves of grapevines after extended predarkening showed substantially lower sucrose levels than basal leaves and very small amounts of hexose were detected in either group. This suggests that considerable sink demand for hexose and also sucrose was imposed on the system by the dark period. Rapid enzymic conversion of sucrose to hexose is guaranteed by high sucrose synthase and invertase activities at least in growing material and early in the season. Persisting, albeit low, photosynthetic rates and stable invertase activities in mature leaves throughout maturation and after harvest are taken as indicative of the latent assimilatory competence of basal leaves to sustain maintenance metabolism and contribute to the perennial carbohydrate storage pool of the vine.

Physiologische Veränderungen in Blättern von *Vitis vinifera* L.: CO_2-Assimilationsraten, Zuckergehalt und Aktivität saccharosespaltender Enzyme

Z u s a m m e n f a s s u n g : Im Verlauf einer Vegetationsperiode wurden die Veränderungen der Photosyntheseraten, des Zuckergehalts sowie der Aktivität saccharosespaltender Enzyme (Invertase und Saccharose-Synthase) in jungen (apikalen) und ausgewachsenen (basalen) Blättern der Weinrebe verfolgt. Die photosynthetische Aktivität basaler Blätter überwog vor Beginn der Beerenreife, sank dann aber auf niedrige Werte ab während apikale Blätter keine entsprechenden Schwankungen zeigten, wodurch ihre Assimilationsrate ab Zeitpunkt Reifebeginn diejenige der basalen deutlich übertraf. Die hohe Photosyntheseleistung in jungem Materiel dürfte auf einen ausgeprägten Bedarf für Assimilate in blatteigenen und benachbarten Verbrauchergeweben zurückzuführen sein.

Die täglichen Schwankungen der Saccharose-, Glucose- und Fructosemengen in apikalen und basalen Blättern waren einander sehr ähnlich. Ungeachtet der geringeren Photosyntheserate zeigten junge Blätter zu Saisonbeginn tendenziell etwas höhere Saccharosewerte als ausgewachsene, ein Zustand, der sich aber nach Reifebeginn ins Gegenteil umkehrte. Allgemein waren niedrige Saccharosegehalte korreliert mit hohen Hexosekonzentrationen und umgekehrt. Hohe Saccharosewerte wurden beobachtet unter Bedingungen, die für das betreffende Organ einen Kohlenstoff-Import oder aber eine Exportbeschränkung erwarten ließen.

Im Verleich mit der als Normalfall einzustufenden Situation vor Beginn der Beerenreife, enthielten apikale Blätter nach einer ausgedehnten Dunkelperiode bedeutend weniger Saccharose als basale. Beide Gruppen wiesen zudem sehr tiefe Hexosepegel auf. Dies deutet darauf hin, daß die Verdunkelung bei unverminderter Nachfrage zu einer Verknappung an Kohlenhydraten geführt haben muß. Ein rascher Stoffwechsel von Saccharose ist zumindest in jungem Material und in der ersten Saisonhälfte garantiert durch hohe Umsatzraten der saccharolytischen Enzyme. Persistierende, wenn auch bedeutend niedrigere Photosynthese- und Invertase-Aktivitäten in basalen Blättern sogar über den Erntezeitpunkt hinaus, werden als Hinweis auf eine weiterhin vorhandene assimilatorische Kompetenz dieser Blätter interpretiert, die zur Aufrechterhaltung des Betriebsstoffwechsels sowie zur Anlage von Reserven dienen kann.

K e y w o r d s : *Vitis vinifera*, leaf, diurnal changes, developmental stages, CO_2 assimilation, sugars, acid invertase, sucrose synthase.

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Introduction

Proper canopy management of the grapevine not only increases photosynthetic activity of leaves, but also unequivocally improves yield as well as grape and wine quality (KOBLET 1984; KLIEWER and BLEDSOE 1987; SMART *et al.* 1990; HUNTER and VISSER 1988b, c). However, the nature of the relationship between canopy management and grape quantity and quality is still unclear, giving rise to many questions concerning the role of different leaf age groups in carbon allocation as the vegetative season progresses.

Partitioning of assimilates between sites of production in photosynthesizing leaves, sites of accumulation in perennial storage compartments, and sites of utilization in the crop, primarily determines yield and longevity of grapevines. Sucrose is the primary photosynthetic product and the major translocated carbohydrate in grapevines (SWANSON and EL-SHISHINY 1959; KOBLET 1977; GLAD *et al.* 1992).

In source leaves, availability of sucrose is regulated by the rate of photosynthesis, sucrose synthesis, and transfer of sucrose to and loading into the phloem (GIAQUINTA 1983). In sinks, the physical constraint (sink size), physiological constraint (sink activity) and the existence of a sucrose gradient at the point of unloading may regulate the rate of import (HO 1988). Here, the sucrolytic enzymes, acid invertase (EC 3.2.1.26) and sucrose synthase (EC 2.4.1.13), are believed to play a major role in converting imported sucrose to hexose (SUN *et al.* 1992). Apparently, wall associated as well as soluble forms of invertase exist, with the latter being the most abundant and presumably active form, particularly in grapes and young leaves of less than half their final size (RUFFNER *et al.* 1990).

Although gradual differences may exist (YANG and HORI 1980), it is well established that grapevine leaves change from assimilate importers to exporters when they reach approximately 50 % of their final size (KOBLET 1969). It was found that apical leaves on main shoots of vines displayed highest rate of photosynthesis (HUNTER and VISSER 1988 a). Basal leaves just above bunches were primarily used to nourish bunches during the whole growth period. Because of leaf age differences, the import/export ratio in canopies of field-grown grapevines continuously changes. Leaves are also exposed to constantly changing microclimates. The fruit itself has a directional bearing on the export status of leaves, as demonstrated by many translocation studies showing a predominant movement of photosynthates towards the grape once a certain fruit size is attained (HALE and WEAVER 1962; QUINLAN and WEAVER 1970; KRIEDEMANN 1977; HUNTER and VISSER 1988 a, b). The above indicates a very complex carbon allocation system in which high demands are imposed on the leaves.

Grapevine canopy management experiments in which the size of the source relative to the sink was reduced, indicated that all leaves had the potential to photosynthesize at a higher rate than the actual (BUTTROSE 1966; MAY *et al.* 1969; KLIEWER and ANTCLIFF 1970; KRIEDEMANN 1977; HOFÄCKER 1978; JOHNSON *et al.* 1982; HUNTER and VISSER 1988 b, c). GOLDSCHMIDT and HUBER (1992) concluded that in mature leaves of a range of species distinguished by different leaf carbohydrate storage mechanisms, sucrose is probably not directly responsible for end-product inhibition of photosynthesis, but regulation is exerted via sucrolysis by acid invertase activity. According to WANG *et al.* (1993) the cleavage of sucrose must be considered a key process in regulating the import of carbon to the fruit.

The ultimate goal of grapevine canopy management is to change carbon allocation to the benefit of fruit sinks without disturbing growth and development in other sinks. More knowledge of diurnal and seasonal regulatory processes and import/export kinetics in leaves of different ages is therefore needed to obtain a better understanding of carbon accumulation and transport within the grapevine. This is paramount to refinement of canopy management recommendations aimed at an integral strategy of optimal production, grape quality and reserve accumulation.

The present study is geared to elucidate the changing import/export status of leaves and to determine the contribution of different leaf groups to various sinks at specific phases during the growth season.

Materials and methods

First group of experiments: Photosynthesis and sugar content

Plant material: *Vitis vinifera* L. cv. Cabernet Sauvignon (clone CS 46) grafted onto 99 Richter (clone RY 30) was used. The vineyard is situated at the experimental farm of the Nietvoorbij Institute for Viticulture and Oenology (Nietvoorbij) at Stellenbosch in the Western Cape. Vines were grown on a Glenrosa (Kanonkop series 13) soil (MACVICAR *et al.* 1977), spaced 3.0 m x 1.5 m and trained onto a 1.5 m slanting trellis as described by ZEEMAN (1981). Water was supplemented by sprinkler irrigation according to A pan evaporation figures on a weekly basis during the growth season; a crop factor of 0.3 was used. Berry set was defined as the stage where berries were 3-4 mm in diameter, while the diameter of berries at pea size was 8-10 mm. Veraison corresponded to full colour break, ripeness to 23-24 °B and post-harvest to 1 month after harvest.Viticultural practices such as suckering and pest and disease control were applied during the growth season according to recommendations by Nietvoorbij.

Photosynthesis measurements: Rate of photosynthesis (mg CO_2 dm^{-2} h^{-1}) was measured in the vineyard with an ADC portable photosynthesis meter (The Analytical Development Co. Ltd., England). The apparatus comprised an infra-red CO_2 analyzer, a data logger, a Parkinson broad leaf chamber and air supply unit. Volume of the chamber was 16 cm^3 and the area 6.25 cm^2. The length of the air supply tube was 4 m. Air flow rate through the open system was adjusted to 300 cm^3/min. Leaves in apical (top 6 leaves) and basal (first 3 leaves above the bunches) positions on the shoot were measured at 2.5 h intervals from 08:00 until 18:00 at berry set, pea size, veraison, ripeness and post-harvest stages. Seven replications, comprising 1 vine per plot, were measured for each leaf position.

Sampling: Apical (first 6 leaves, starting at the first unfolded lamina) and basal (first 3 leaves above the bunches) leaf blades on a shoot were sampled at 2.5 h intervals from 08:00 until 18:00, kept dark, transferred to the laboratory and processed immediately. Leaf groups were sampled in triplicate. Sampling was done at berry set, pea size, veraison, ripeness and post-harvest stages.

Extraction of sucrose and hexoses: A modified method of RUFFNER *et al.* (1990) was used. Leaf material was cut to approximately 1 cm^2 pieces after removal of the main veins. Batches of 2 g fresh weight (F.W.) tissue were homogenized in liquid N_2 using a mortar and pestle. The powder was suspended in 50 ml MeOH - $CHCl_3$ - 0.2 M HCO_2H (12:5:3 v/v) (pH approximately 4.2) and soluble sugar extracted for 30 sec at 20500 rpm with an Ultra-Turrax macerator. The homogenate was transferred to a sintered glass filter and further extracted with two 25 ml volumes of 80 % ethanol. Filtrates were combined, taken to dryness in a rotary evaporator at 35 °C and the residue redissolved in 5 ml of 50 % acetonitrile.

Sugar analyses: Sugars were analyzed by HPLC according to HUNTER *et al.* (1991).

Second group of experiments: Sugar and enzymes

Plant material: *Vitis vinifera* L. cv. Müller-Thurgau (Riesling x Silvaner), spaced 1.0 m x 1.8 m and vertically trained, was used. The vineyard is situated next to the Zürich Botanical Gardens. Vines were grown under non-irrigated conditions. Pest and disease control were performed as needed. Twelve selected vines at berry set were completely covered with a black polyethylene sheet for 36 h (night-day-night), whereafter the sheet was removed and the first samples taken immediately.

Sugar extraction and analyses: Sugars were extracted as above and analyzed using an HPLC system consisting of a Gynkotek model 480 pump, Knauer RI detector and Shimadzu C-R5A Chromatopac integrator. A Spherisorb NH_2 column (125 mm x 4 mm ID; 3 µm particle size) and guard column (20 mm x 4 mm ID) with the same packing were used. The column was operated at room temperature and sugars separated with 82 % acetonitrile at a flow rate of 0.5 ml/min.

Extraction of enzymes: Leaves (2-4 g F.W.) were homogenized for 2 x 1 min (Sorvall Omnimixer) in 50 ml bis-tris propane buffer (0.15 M), containing 1 % (w/v) polyethylene glycol 4000, 10 mM $MgSO_4$, 5 mM dithiothreitol and 100 mM cysteine/HCl, pH adjusted to 7.4 with NaOH. The crude homogenate was centrifuged at 17 000 x *g* for 10 min, 2.5 ml of the supernatant desalted over a Pharmacia PD-10 column and the eluent used directly for determination of enzymic activity.

Assay for sucrose synthase activity (sucrose degrading direction): 100 µl MES (4-morpholineethane-sulfonic acid)/NaOH-buffer (0.2 M), 100 µl 0.8 M aqueous sucrose solution, 100 µl sample, and 100 µl UDP (uridine-5'-diphosphate: 15 mM) were pipetted sequentially into a test tube. A blank without UDP was set up as reference. The mixture was incubated at 30 °C for 20-30 min. The reaction was stopped by immersing the test tubes in boiling water for 90 sec. The samples were then immediately cooled to room temperature and UDP-glucose determined in 100 µl of the mixture after adding 800 µl 2.5 mM NAD in glycinate/NaOH-buffer (0.5 M, pH 8.7) and finally 10 mU of UDPG-DH (Sigma Type III). Reduction of NAD was measured at 340 nm in a double beam spectrophotometer.

Invertase assay: 100 µl of desalted extract was incubated in a mixture consisting of 100 µl 0.4 M aqueous sucrose solution and 200 µl NaOAc-buffer (0.2 M, pH 4.0) for 20 min at 30 °C. The reaction was stopped by addition of 0.5 ml DNSA-reagent) 1 % 3,5-dinitrosalicylic acid (w/v) in 0.5 M KOH and 1 M K/Na-tartrate), thus shifting the pH far into the alkaline range. After supplementing the assay with 0.5 µmol fructose to avoid oxygen interference at low reducing sugar concentrations, the mixture was kept in boiling water for 10 min, cooled to room temperature and colour intensity read at 560 nm against a blank with zero reaction time. Enzymic activity is expressed as nmole sucrose hydrolyzed per sec (nkat) under the above conditions.

Statistical analyses: Where applicable, data were analyzed with a SAS program. Student's t-LSD was used to test significant differences among treatment means.

Results and discussion

In basal leaves CO_2 assimilation increased until the berries reached pea size, whereafter it declined to low rates during the ripening and post-harvest periods (Fig. 1). This is consistent with results found by PANDEY and FARMAHAN (1977). The apical leaves in this study displayed a more or less uniform rate of photosynthesis throughout the season, increasing only slightly at ripeness and decreasing after harvest. It is assumed that basal leaves represent the photosynthetic pattern during the season more specifically, because identical leaves were always measured regardless of shoot elongation. The term "apical leaves", on the other hand, is related to shoot growth. Regardless of leaf position, a general decline in photosynthesis was noticed as the season advanced. Similar results were found by KRIEDEMANN (1977) and HUNTER and VISSER (1988 a, b, c) and were related to senescence of leaves, seasonal conditions, changing demands from sinks including the crop, and modifications of canopy and leaf exposure. Generally, basal leaves showed higher photosynthetic activity than apical leaves until pea size, whereafter this pattern was reversed. Although chlorophyll content is not regarded a reliable index of photosynthetic activity (KRIEDEMANN *et al.* 1970; HUNTER and VISSER 1989), the results correspond to leaf chlorophyll concentrations (HUNTER and VISSER 1989) and seem to reflect primarily the influence of leaf senescence. Interesting, however, is the continued CO_2 assimilation of basal leaves, albeit at a low rate, even after removal of the fruit sinks. According to CANDOLFI-VASCONCELOS and KOBLET (1990), leaves on the major shoot are mainly responsible for sugar supply to the fruit and to reserve organs during the first half of the season, while

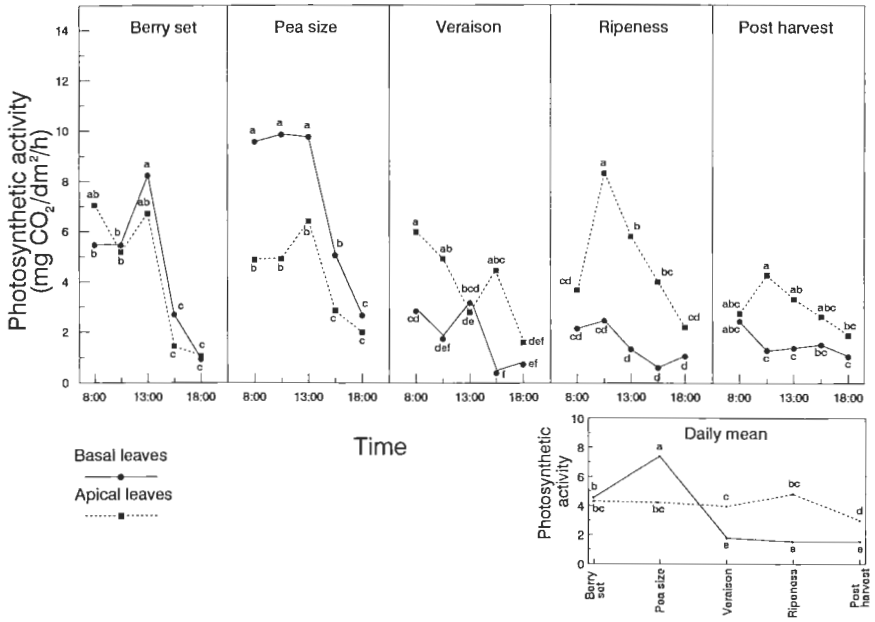


Fig. 1: Diurnal photosynthetic activity of basal and apical leaves of Cabernet Sauvignon vines at different stages of grape development. Values followed by the same letter do not differ significantly ($p < 0.05$) for each developmental stage. Daily mean values were compared over all stages.

during the ripening period photosynthates are mainly supplied by leaves on lateral shoots, which are more or less comparable to the apical leaves in this study. Source reduction by partial defoliation during the pre-pea size period also showed that, although leaves were highly stimulated photosynthetically (HUNTER and VISSER 1989), grape yield was severely reduced (CANDOLFI-VASCONCELOS and KOBLET 1990; HUNTER and VISSER 1990 b). Retaining of mature leaves situated on the lower half of the shoot in the pre-pea size period is therefore needed for supplying carbon to sinks such as the roots, trunk, shoots and fruits (cf. also BUTTROSE 1966; KLIEWER and FULLER 1973). In the light of their continued production of photosynthates even after harvest, basal leaves undoubtedly play an important role in reserve accumulation after harvest. Removal of these leaves (e.g., to facilitate easier harvesting) would therefore evidently affect reserve accumulation, which may have implications for the next season's production.

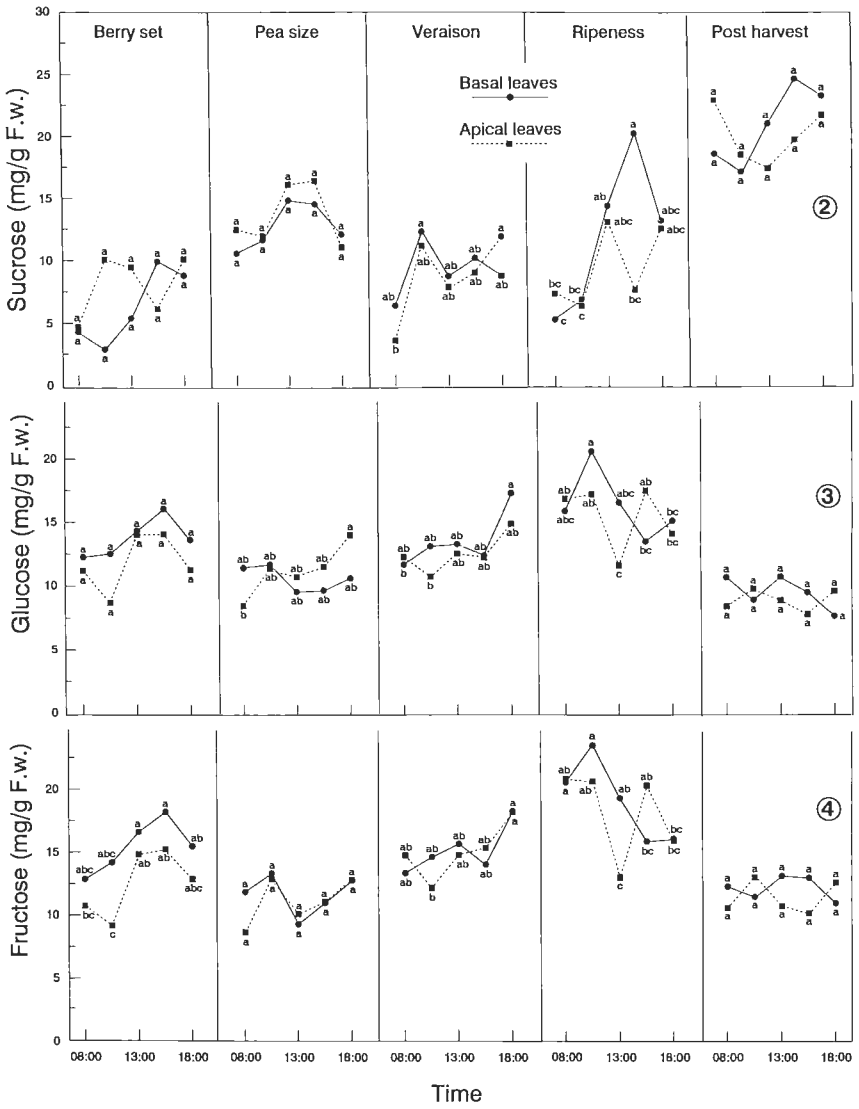
Diurnally, highest rates of photosynthesis generally occurred before 13:00, whereafter it decreased to low levels at 18:00. The relatively high photosynthetic rate at 08:00 is noteworthy. A decrease in ambient light intensity in particular, undoubtedly played a significant role in the decline of photosynthesis in the late afternoon.

In basal and apical leaves of Cabernet Sauvignon, similar fluctuations in sucrose (Fig. 2), glucose (Fig. 3) and fructose (Fig. 4) concentrations were observed throughout the season. The pattern for sucrose, however, was opposite to the behaviour of glucose and fructose, increasing from berry set until the post-harvest stage. KLIEWER (1966) similarly found sucrose to increase in leaves, shoots and roots, from green berry stage until the overripe stage.

Average hexose concentrations were lower in apical than in basal leaves during the whole growth period (Figs. 3 and 4). Sucrose, on the other hand, was higher in apical leaves until the berries reached pea size; afterwards sucrose in basal leaves was predominant (Fig. 2). This is directly opposite to the measured rates of photosynthesis (Fig. 1). However, it may be indicative of high export rates in basal leaves. Being recently matured, they are able to sustain sucrose export while still maintaining a high sucrose content (Fig. 2) because of their high photosynthetic rates, at least up to "pea size" (Fig. 1).

In contrast, apical leaves most probably hoarded photosynthates for their own growth and development during this period as was shown previously (HUNTER and VISSER 1988 a), resulting in high sucrose contents and comparatively low photosynthetic rates. This is not consistent with the very small amount of free sucrose found in leaves during the import stage (GIAQUINTA 1983; RUFFNER *et al.* 1990). Nevertheless, it is important that during the pre-pea size period the first six leaves sampled in this study comprised a spectrum from just unfolded leaves with import characteristics up to laminae approximately one third of their final size (data not shown). Because the elongation of shoots virtually stops at veraison, leaves of the apical group would have grown to 50 % of their final size before analysis and should therefore rather be classified as having an intermediary import/export status during the latter part of the growth season.

Except for the post-harvest stage, hexose concentrations were generally higher than sucrose concentrations. The rise in sucrose concentration and decline in glucose and fructose concentration at pea size correspond to the lag phase in berry growth (phase III according to ALLEWELDT 1977). A decrease in demand for sucrose, at least from the developing berry, as well as reduced sucrolysis would thus be expected during this period. The elevated sugar concentrations in the leaves at ripeness probably supplied in demands from fruit and storage sinks (shoots, trunk, roots)

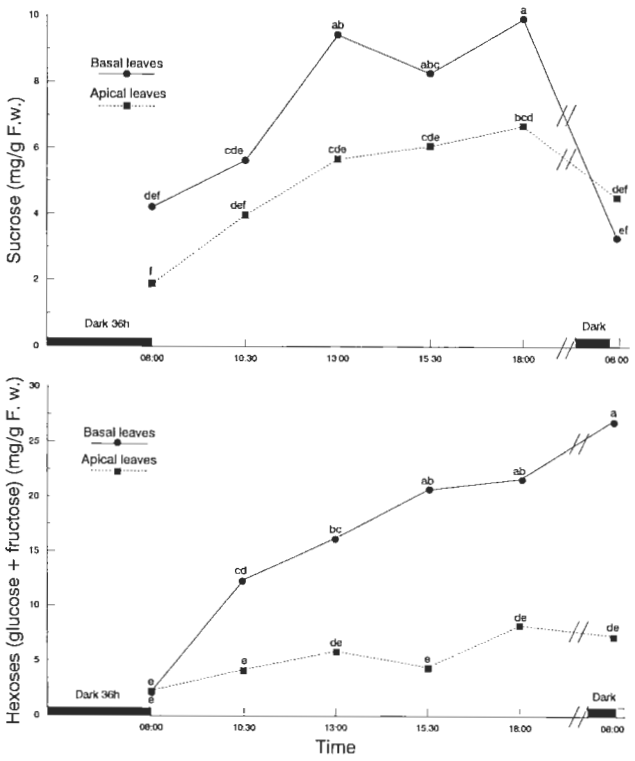


Figs. 2–4: Diurnal sucrose (Fig. 2), glucose (Fig. 3), and fructose (Fig. 4) concentrations in basal and apical leaves of Cabernet Sauvignon vines at different stages of grape development. Values followed by the same letter do not differ significantly ($p < 0.05$) for each developmental stage.

in particular. Hunter and Visser (1988 a) showed that a redistribution of photosynthates to vegetative organs is likely to take place at berry ripeness and that bunches were mainly supported by basal leaves. After harvest, glucose and fructose concentrations decreased to levels similar to those at the pea size stage (Figs. 3 and 4), whereas sucrose concentrations continued to increase (Fig. 2), probably because reserve accumulation was favoured during this period (Kriedemann 1977). The corresponding fall in photosynthetic rate (Fig. 1) which occurred particularly in basal leaves from veraison onwards appears interesting. The fact that this also occurred in apical leaves at the post-harvest stage would, in contrast to the findings of Goldschmidt and Huber (1992), indicate some inhibitory relationship between sucrose accumulation and photosynthetic rates. Although there is some controversy about the effect of carbon translocation and assimilate demand on the process of photosynthesis (Neales and Incoll 1968; Wareing *et al.* 1968; Geiger 1976; Hansen 1977; Acock *et al.* 1990; Goldschmidt and Huber 1992), it stands to reason that under favourable conditions, export of as well as demand for sucrose would increase its production (Hawker *et al.* 1991). However, the nature of the control mechanism still remains open to speculation (Eschrich and Eschrich 1987).

No definite diurnal pattern was found for either the monosaccharides or the disaccharide. However, whilst for sucrose 18:00 concentrations were almost always higher than 08:00 concentrations, glucose and fructose concentrations followed this pattern only until veraison, whereas later in the season hexose levels decreased or remained constant. Davis and Loescher (1991) also found a sharp increase in sucrose levels during the afternoon in mature and old celery leaves. The afternoon increase in sucrose concentration (Fig. 2) probably contributed to a concomitant decline in photosynthetic rate (Fig. 1). The present results suggest that sucrose was metabolized and exported at a slow rate after berry ripeness and accumulated in leaves during this period.

After 36 h predarkening of whole Müller-Thurgau vines at berry set, actively growing apical leaves initially showed



Figs. 5 and 6: Photosynthetic replenishment of sucrose (above) and hexose (below) pools in basal and apical grapevine leaves after extended predarkening. Values followed by the same letter do not differ significantly ($p < 0.05$).

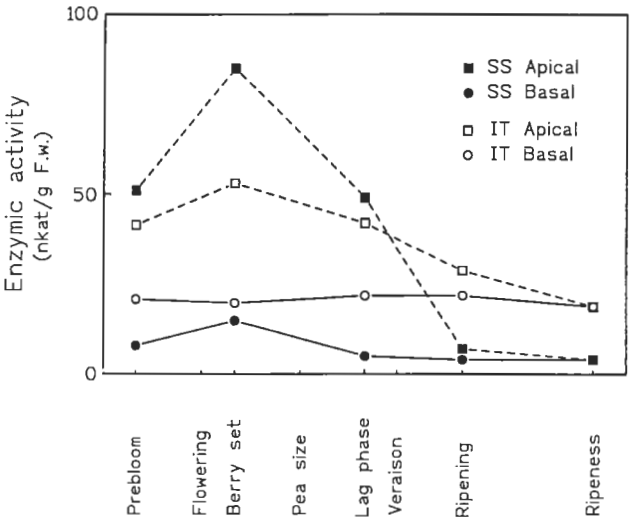


Fig. 7: Sucrolytic activities of sucrose synthase (SS) and invertase (IT) in basal and apical leaves of Müller-Thurgau grapevines at different stages of grape development.

a substantially lower sucrose level than mature leaves (Fig. 5) or either group of leaves from untreated plants (Fig. 2). This clearly indicates that locally photosynthetised as well as sucrose imported from neighbouring leaves contribute to the sucrose levels (4 mg/g F.W.) normally found in apical leaves (HUNTER and VISSER 1988 a). The hexose contents of all leaves were reduced after darkening to a fraction of their normal values (Fig. 6). Hexose recuperation in apical leaves occurred very slowly, presumably as a result of assimilate consumption for growth, while sugar concentrations in basal leaves returned to normal in the course of one day. Interestingly enough, sucrose levels in either group of leaves dropped to less than 5 mg/g F.W. during the following night, thus emphasizing the translocatory role of this component (SWANSON and EL-SHISHINY 1959).

The complementary pattern of sucrose versus glucose/fructose concentrations observed throughout the season (Figs. 2, 3, 4) indicates a non-equilibrium situation for sucrolysis (RUFFNER and HAWKER 1977). The very low hexose levels found after predarkening (Fig. 6), which are correlated with normal (basal leaves) and low sucrose contents (apical leaves), respectively, point in the same direction. An investigation of the sucrolytic enzymes sucrose synthase and invertase (Fig. 7) confirms that substantial differences existed between the activities of these enzymes in young and maturing leaves at the time of the pre-darkening experiment (pre-veraison). The observed high activities of both enzymes in apical leaves before the onset of ripening would explain the low sucrose concentrations found in this material after predarkening, provided that metabolic demand for assimilates was exerted via the hexose pool. However, hexose levels in growing and mature leaves fluctuated practically in tandem throughout the vegetation period, irrespective of changing enzymic activities. Furthermore, the decrease in sucrolytic activity observed after veraison had no immediate bearing on the hexose contents. It is therefore believed that the present results support the classic concept of an involvement of sucrose synthase (HO 1988) and invertase (RUFFNER *et al.* 1990) in assimilate import and consequently foliar growth, which is known to be severely reduced after veraison (HUNTER and VISSER 1990 a).

Elevated sucrose levels were consistently found in leaves when export was limited, either due to an intrinsic incompetence of the tissue to export assimilates (apical leaves), the known lag-phase in berry growth (ALLEWELDT 1977) causing reduced sink demand (basal leaves at pea size), removal of sinks (harvest) or slow filling of distant sinks (roots, post-harvest). On the other hand, a uniform invertase pool of approximately 20 nkat/g F.W. was invariably present in mature leaves throughout the vegetative period and sucrolytic activity in young leaves dropped to the very same level at the end of the season. Conceivably, this residual invertase represents a form of the soluble enzyme which is responsible for the conversion of sucrose to hexose, which is needed for metabolic maintenance processes in the photosynthetically fully competent leaf after loosing its sink properties.

It must be emphasized that the two vineyards used for sucrose and enzyme determinations were cultivated under different climatic and agricultural conditions. Although it is expected that physiological patterns would be similar, results might not be directly comparable. However, field studies at a biochemical level provide new perspectives on environmental effects and the integrative behaviour of whole plants, which is of particular interest when studying crop plants. The data are believed to contribute to our understanding of the dynamics of photosynthesis and carbohydrate metabolism in grapevine foliage as affected by leaf position and developmental stage of the vine.

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