

Photosynthetic duration, carboxylation efficiency and stomatal limitation of sun and shade leaves of different ages in field-grown grapevine (*Vitis vinifera* L.)

by

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S u m m a r y : The relationship of photosynthesis (A) of grapevine (*Vitis vinifera* L.) sun and shade leaves of primary and secondary (lateral) shoots to insertion level was investigated over two seasons in the field. The leaf plastochron index (LPI) was used to denote leaf position on the shoot. Additionally, laboratory and field measurements of the response of A to CO_2 were conducted. An empirical model was developed to estimate carboxylation efficiency (CE) and stomatal limitations (l) of A . In sun leaves, the relationship of A to LPI changed little until the end of the season (October), whereas stomatal conductance (g) and the intercellular partial pressure of CO_2 (c_i) increased. Leaves acclimated to low light and leaves older than LPI 5 had 30 % lower A and were operating at a slightly higher c_i as sun leaves. During September and October, lateral leaves had highest rates of CO_2 assimilation and CE. In mid-October, A and g decreased rapidly and simultaneously for all leaf types, leaf positions and both treatments (sun and shade). Photosynthesis responded similar to individual leaf age as to leaf position. A was linearly related to c_i up to non-limiting conductances for sun and shade leaves, for all ages and at all times during the season. The CE and l were highest at the beginning of the season and strongly dependent on leaf position. Stomatal limitation declined continuously from about 55 % at the beginning to about 23 and 18 % for sun and shade leaves, respectively, at the end of the season.

K e y w o r d s : Vitaceae, leaf age, sun, shade, photosynthesis, stomatal limitation, carboxylation efficiency, empirical model, primary shoots, secondary shoots.

A b b r e v i a t i o n s : A = photosynthetic rate, g = stomatal conductance for CO_2 , g_a = boundary layer conductance for CO_2 , g_{tc} = total (stomatal and boundary layer) conductance for CO_2 , g_H = stomatal conductance for H_2O , PFD = photon flux density, c_a = ambient partial pressure of CO_2 , c_i = internal partial pressure of CO_2 , CE = carboxylation efficiency, Γ = CO_2 compensation point, LPI = leaf plastochron index, l = stomatal limitation of photosynthesis, T_l = leaf temperature.

Introduction

Concomitantly with the structural development of a leaf and its chloroplasts, photosynthetic function begins. Initially, as leaves expand, they are sustained by imported carbon and accomplish little net photosynthesis. Maximum photosynthetic activity under optimal conditions and ambient CO_2 concentration is typically reached at or slightly before the time when leaves reach full expansion (KRIEDEMANN *et al.* 1970; ALLEWELDT *et al.* 1982; CATSKY and TICHÁ 1980). As leaves age further, photosynthetic capacity, stomatal conductance (KRIEDEMANN 1968; SCHULZE and HALL 1982), leaf dry mass per area (WERMELINGER and KOBLET 1990; PONI *et al.* 1994 b), nitrogen (WERMELINGER and KOBLET 1990; PONI *et al.* 1994 b), protein (BETTNER *et al.* 1986), and RNA contents (THOMAS and STODDART 1980), and the activity of several sacrolytic (HUNTER *et al.* 1994) and photosynthetic enzymes including ribulose-1.5-bisphosphate carboxylase (Rubisco) decrease (HUNTER *et al.* 1994; ZIMA and ŠESTÁČ 1979).

Depending on the plant species, these changes may occur simultaneously or phase-delayed and thus govern whether stomatal or non-stomatal components are more

limiting photosynthesis during aging and whether the limiting component changes during a growing season, respectively a plant's life (SOLÁROVÁ and POSPISOVÁ 1983; DÜRING 1994). For plants which form complex heterogeneous canopies, such as grapevines, differences in light exposure during the development of single leaves may modify the photosynthetic apparatus and alter patterns of photosynthesis during leaf development and aging (JURIK *et al.* 1979, CARTECHINI and PALLIOTTI 1995). Understanding of the limitations and the contribution of photosynthesis of sun and shade leaves of different age and at different times during a season is important for modelling whole-plant assimilation, which can be substantially over- or underestimated if the effects of leaf age and light exposure are ignored (HODÁNOVÁ 1979, CALDWELL *et al.* 1986).

To provide the basis for modelling grapevine photosynthesis, the objectives of the present study were: 1. to compare changes in maximum photosynthetic activity (under ambient CO_2) of grapevine sun and shade leaves during the season related to leaf position on the shoot (leaf age structure) with changes related to individual leaf age history; 2. assess for the possible limitations of photosynthesis in sun and shade leaves during aging.

Material and methods

Field experiments: Experiments were conducted over two years (1987-1988) on 9-year-old field-grown White Riesling grapevines (*Vitis vinifera* L.) clone 198 Gm on 5C rootstocks at the State Research Institute in Geisenheim, Germany (50° North, 8° East). Details of growing conditions and soil analysis are given elsewhere (SCHULTZ 1990). Vines were pruned to 10 buds/m² in January. Vineyard management was according to commercial practices with the exception that shoots remained unhedged throughout the season. Sun shoots were defined as shoots growing on the canopy exterior well exposed to light throughout most of the day. Shade shoots grew in the canopy interior receiving less than 12 mol·m⁻²·d⁻¹ (photon flux density, PFD) integrated over a photoperiod. By definition, shade shoots were present only after canopy closure, about 1 week before bloom.

Laboratory experiments: In addition to the field studies in Germany, some experiments were conducted under semi-controlled conditions at the University of California, Davis, USA. For this purpose, two separate batches of 4-year-old grapevine plants (*Vitis vinifera* L. cvs Zinfandel and White Riesling) were grown in the greenhouse (April to August) or outdoors (August to December) in 1991 and 1992 in 25 l pots containing a soil:peat:perlite mixture (1:3:3). Growth conditions in the greenhouse were 25-30 °C day and 18-24 °C night temperature, a relative humidity of > 50 % and a 10-15 h photoperiod. Plants were grown outdoors late in the year to simulate temperature

conditions encountered by field plants during the pre-harvest period in Germany.

In each experiment, eight plants were grown under full sunlight (> 1400 μmol·m⁻²·s⁻¹ (maximum PFD); >16 mol·m⁻²·d⁻¹ integrated PFD as determined with a LI-COR 190s Quantum Sensor) and eight plants were grown under a neutral shade screen (< 300 μmol·m⁻²·s⁻¹ (maximum PFD) and < 10 mol·m⁻²·d⁻¹ integrated PFD). Approx. 1 week after bud break, all plants were thinned to 2 shoots with at least one inflorescence per shoot.

Physiological age: Physiological leaf age was defined with the leaf plastochron index (LPI). The plastochron concept (ERICKSON and MICHELINI 1957) is suitable for the description of age-related changes in sun and shade leaf photosynthesis of grapevines under field conditions (SCHULTZ 1993). Leaves on secondary, lateral shoots were classified into apical (upper 3-5 leaves on a shoot) and basal leaves only.

Field gas exchange measurements: Gas exchange was measured with an open-system gas exchange apparatus (Walz, Effeltrich, Germany) during 6 phenological phases: PP I, bud-break-bloom; PP II, bloom-berry pea size; PP III, berry pea size-end of berry growth phase I; PP IV, veraison until 1 week after veraison; PP V, mid-maturity (3-5 weeks after veraison); PP VI, 1 week prior to harvest to harvest. Measurements were conducted between 9 a.m. and 12 a.m. on days with optimal conditions for high rates of photosynthesis (PFD, >1400 μmol·m⁻²·s⁻¹; optimum leaf temperatures; and non-limiting vapour pressure deficit (for further details see

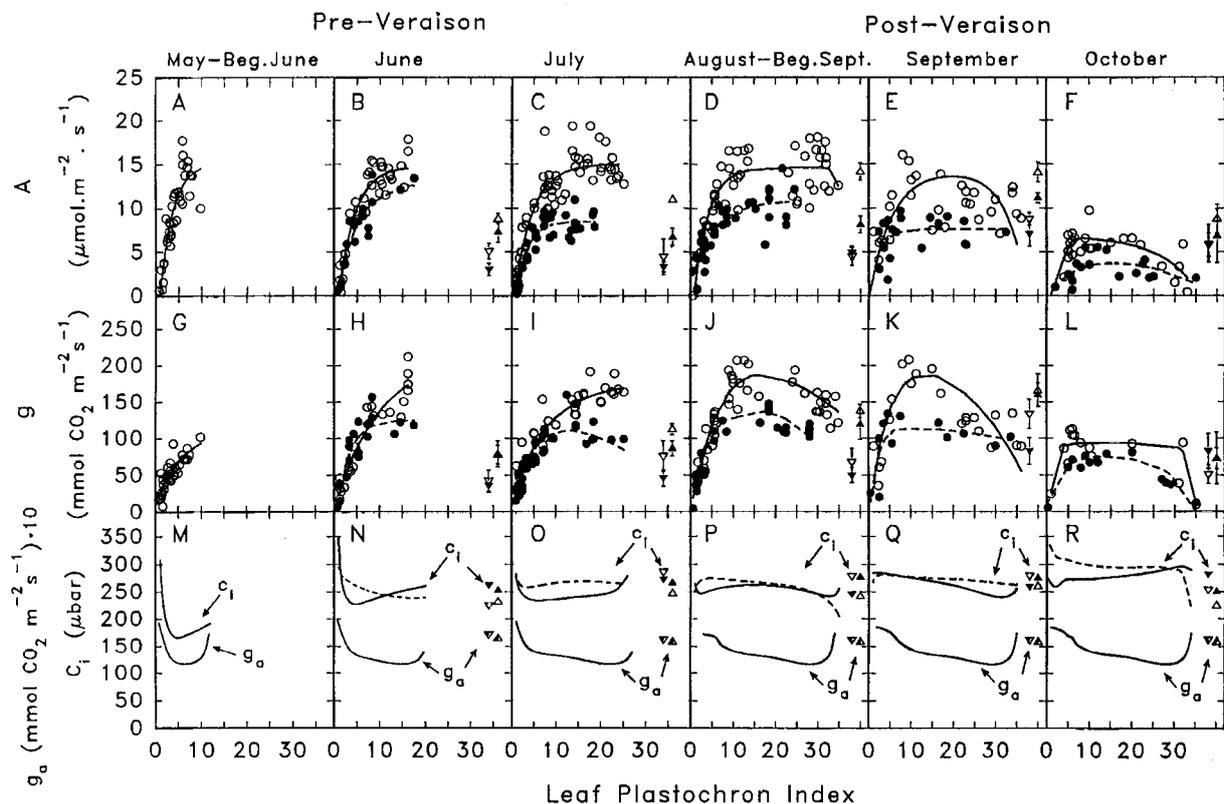


Fig. 1: Relation of photosynthesis (A-F); stomatal conductance for CO₂ (G-L); boundary layer conductance and intercellular CO₂ concentration (M-R), to LPI, i.e. leaf position on the shoot, for 3 pre-veraison and 3 post-veraison phases. Circles and triangles represent values from leaves on primary and lateral shoots (○ apical, △ basal). Values for laterals represent the mean ± SE of 5-7 leaves. Open symbols and continuous lines denote sun leaves, closed symbols and dashed lines denote shade leaves.

SCHULTZ 1990). Average leaf temperatures (°C) were: (PP I) 27.8; (PP II) sun, 27.7, shade, 27.0; (PP III) sun, 27.8, shade, 26.9; (PP IV) sun, 27.2, shade, 26.3; (PP V) sun, 22.2, shade, 22.5; (PP VI) sun, 19.4, shade, 19.3. Ambient partial pressure of CO₂ (c_a) during measurements was typically 345 ± 10 µbar as determined with an URAS-2 infrared gas analyser (Hartmann & Braun, Germany) in an adjacent vineyard. Boundary layer conductance for CO₂ of leaves of different age, and hence different size, was estimated from leaf length and leaf width and calculated for a wind speed of 4 m·s⁻¹ according to GATES (1980). There were no consistent differences in the sizes of sun and shade leaves. All gas exchange parameters were calculated using the equations of VON CAEMMERER and FARQUHAR (1981).

To determine the dependence of photosynthesis and stomatal conductance for CO₂, on leaf age structure, i.e. insertion level on a shoot, 10 primary shoots on each of 10 vines were selected at bud break or at canopy closure ("shade shoots") for measurements of gas exchange. Measurements were not conducted on the first, usually malformed, two basal leaves on a shoot. Measurements on lateral leaves were also conducted on these shoots.

The development of A and g during leaf ontogeny of individual sun and shade leaves on primary and lateral shoots was followed on 5-10 leaves per treatment (sun or shade) unfolding at different times during the season. For measurements on shade leaves, shoots growing inside the canopy were temporarily exposed to full sun light (approx. 5-10 min). There was no evidence for photoinhibition caused by this treatment, as judged from the stability of A during the measurements.

L a b o r a t o r y g a s e x c h a n g e m e a s u r e m e n t s: Measurements of the relationship of A to c_i were conducted under saturating PFD in the morning between 8 and 11 a.m. with an open system gas exchange apparatus previously described by SIMS and PEARCY (1989) on potted plants grown in the greenhouse or outdoors. For the determination of the CO₂ compensation point (Γ), the linear portion of the A-c_i curve was extrapolated to the abscissa using linear regression analyses. Measurement series were conducted during PP I-III (pre-veraison) and PP IV-VI (post-veraison). Leaf temperature was controlled at 27.1 ± 0.5 °C, close to the temperature optimum of A for most of the season in the field and vapour pressure deficit between leaf and air was maintained at 6-11 mbar. Air of a given CO₂ partial pressure was obtained by mixing CO₂ free air with air containing 5 % CO₂ using mass flow controllers. The light source was a 1500 W metal halide lamp (Sylvania Metalarc) providing a PFD of up to 1500 µmol·m⁻²·s⁻¹.

E s t i m a t i o n o f s t o m a t a l l i m i t a t i o n o f p h o t o s y n t h e s i s: A simple empirical model was devised to estimate stomatal and non-stomatal limitations to photosynthesis from field measurements of A and g. Data of the relationship of A and g to LPI of sun and shade leaves were pooled into each of the 6 phenological phases (3 pre-veraison, 3 post-veraison) (Fig. 1). For primary shoots, either a 'single' (eq. 1) or a 'double' (eq. 2) exponential equation was then fitted to the data

$$A(g) = A_{\max}(g_{\max}) \cdot [1 - (1/e^{(\alpha \cdot (LPI-\beta))})] \quad (1)$$

$$A(g) = A_{\max}(g_{\max}) \cdot [Z] \cdot [1 - (1/e^{(\gamma \cdot (LPI_{\max} - LPI))})] \quad (2)$$

$$Z = 1 - (1/e^{(\alpha \cdot (LPI-\beta))})$$

where A_{max} and g_{max} represent maximum values of A and g, and α, β, γ and LPI_{max} are parameters which describe the shape of the curve. For lateral shoots, leaves were only roughly divided into apical (upper 3-5 leaves) and basal locations and average A and g values determined. The c_i as a function of LPI was estimated from A and g calculated by equations 1 and 2 according to

$$c_{i(LPI)} = c_a - A/g_{tc} \quad (3)$$

where c_a was 345 µbar and g_{tc} is the total (stomatal and boundary layer) conductance for CO₂.

The dependence of Γ on leaf position (LPI) on the primary shoot was described using an exponential equation fitted to data obtained in the laboratory (27.1 °C),

$$\Gamma_{(LPI)} = \Gamma_{\max} \cdot e^{(-k \cdot LPI)} + \Gamma_{\min} \quad (4)$$

where Γ_{max} and Γ_{min} are the maximum and minimum CO₂ compensation points (µbar), respectively, of all leaves on a primary shoot, and k can be termed "age" coefficient. For lateral shoots, only an average Γ was determined for apical and basal leaves.

The temperature dependence of Γ was determined for leaves > LPI 10 during the post-veraison phase in the field with a modified ADC LCA 3 gas exchange system following a similar protocol as described for the laboratory experiments. Assuming that the response of Γ to temperature is similar for all leaf ages, Γ as a function of LPI and T_l could be calculated from

$$\Gamma_{(LPI, T_l)} = \left(\frac{\Gamma_{(LPI)}}{\Gamma_{\min}} \right) \cdot [8.21 \cdot e^{(0.059 \cdot T_l)}] \quad (5)$$

where Γ_(LPI) and Γ_{min} are estimated from equation 4 (for 27.1 °C) and T_l is the actual leaf temperature.

A resistance analog-type sub-model was then used to estimate stomatal limitation to photosynthesis (JONES 1985), based on the linear response of A to c_i for both sun and shade leaves of all ages up to internal CO₂ concentrations where photosynthesis was no longer limited by stomata. Combining the results of eqs. 1-5, the carboxylation efficiency, CE (µmol CO₂·m⁻²·s⁻¹·µbar⁻¹), i.e. the initial slope of the A vs. c_i curve, can be estimated for different LPIs from

$$CE_{(LPI)} = \left[\frac{A}{(c_i - \Gamma)} \right] \quad (6)$$

and stomatal limitation (l) can be calculated for every LPI from

$$l_{(LPI)} = [1 - (A/((c_a - \Gamma) \cdot CE))] \cdot 100 \quad (7)$$

where term (c_a-Γ)·CE calculates the photosynthetic rate without stomatal limitation, and A is calculated from eqs. 1 or 2. Parameter estimates for equations 1, 2, 4, and 5 were obtained by least square non-linear regression analyses using the program PROC NLIN of SAS (SAS Institute 1987).

Results

Effect of leaf position on A , g , g_a and c_i : During the three pre-veraison phenological phases, maximum A and g (stomatal conductance to CO_2) of sun leaves were reached at about LPI 10-15 (Fig. 1 A-C, G-I). For shade leaves, maximum A and g , and the LPI at which these maxima occurred decreased gradually during the same period (Fig. 1 A-C, G-I). There was no decrease in A and g of leaves on basal shoot positions. The g_a was roughly 10 times g for leaves $>$ LPI 6 and reflected changes in leaf size with position on the shoot (Fig. 1 M-R). Stomatal conductance at any leaf age was lowest at the beginning of the season (Fig. 1 G, May - early June), which resulted in lower c_i values as compared to the rest of the growing season. During all three pre-veraison measurement periods, c_i was highest in sun and shade leaves near the shoot apex (near LPI 0) (Fig. 1 M-O), suggesting that the intrinsic capacity for assimilation is small in these young leaves. Older leaves within a light treatment showed little variation in c_i along the shoot (Fig. 1 M-O). In shade leaves, the ratio of A to g was slightly lower than in sun leaves, yielding higher c_i values throughout the season (Fig. 1 M-R). Due to their smaller average leaf size, g_a was generally higher in lateral as compared to most primary leaves (Fig. 1 M-R). Both A and g were lower in lateral as compared to primary leaves until veraison (Fig. 1 A-C, G-I). Lateral shade leaves had generally lower rates of A and g than sun leaves throughout the season.

As the season progressed and shoots reached the maximum number of leaves during the early post-veraison phase (beginning of September), g of sun and shade leaves older than LPI 15 started to decline, whereas a reduction in A was less apparent (Fig. 1 D, J, P). At this time of the season, c_i was almost constant for leaves of all ages and similar for sun and shade shoots (Fig. 1 P). Near the end of September, A and g of basal sun leaves on primary shoots declined (Fig. 1 E, K, Q). During this period of fruit ripening, basal lateral leaves had reached maximum CO_2 assimilation rates exceeding those of most leaves on primary shoots, 14.7 as compared to 13.8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for sun and 10.9 as compared to 8.3 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for shade leaves, respectively (Fig. 1 E).

In October, A and g declined strongly and simultaneously in all leaves on primary shoots, with sun leaf values decreasing proportionally more than shade leaf values (Fig. 1 F, L). The further increase in c_i as compared to earlier stages suggested a substantial loss in assimilation capacity in the mesophyll (Fig. 1 R). In contrast, c_i of lateral leaves did not increase (Fig. 1 Q, R) and the reduction in A was less pronounced (Fig. 1 F, R).

Photosynthesis in relation to individual leaf age history: It was hypothesized that the relationship of A to the individual physiological leaf age was similar to the relationship of A to leaf position. To test this, the development of A at different physiological ages of individual sun and shade leaves of primary and lateral shoots was followed for leaves unfolding at various times between May and September. At each measurement date, the mean leaf age in plastochrons

was noted. Lateral leaves were designated "apical" for a period of 5 plastochrons and "basal" thereafter. Equations 1 and 2 were then used to calculate A using the appropriate parameters previously estimated for each phenological phase from the A vs. LPI relationship (Fig. 1; Appendix).

The comparison of measured (Fig. 2, symbols) and calculated values (Fig. 2, lines) of A of sun and shade leaves of primary and lateral shoots indicated that in grape the response of A to leaf position is similar to the relationship of A to the individual leaf age. Leaves on primary shoots unfolding in May or June rapidly reached high photosynthetic rates and maintained them for $>$ 100 d (Fig. 2 A, B), whereas A of lateral leaves continuously increased over a similar time span (Fig. 2 C). It is also apparent, that the distinction of lateral leaves in "young" and "old" leaves results in sometimes abrupt changes in the estimates of A when the transition between the 2 stages is reached, with each group having different parameter values (Appendix; Fig. 2 C, E, G, I). For all unfolding times, the photosynthetic longevity of the shade leaves was similar to those of the sun leaves.

A - c_i relationship and CO_2 compensation point: With decreasing c_i in the range from 345 μbar to Γ , A decreased linearly for all LPIs, independent of the previous light treatment and time during the season (Fig. 3). Non-linearity of the A - c_i curves started at or

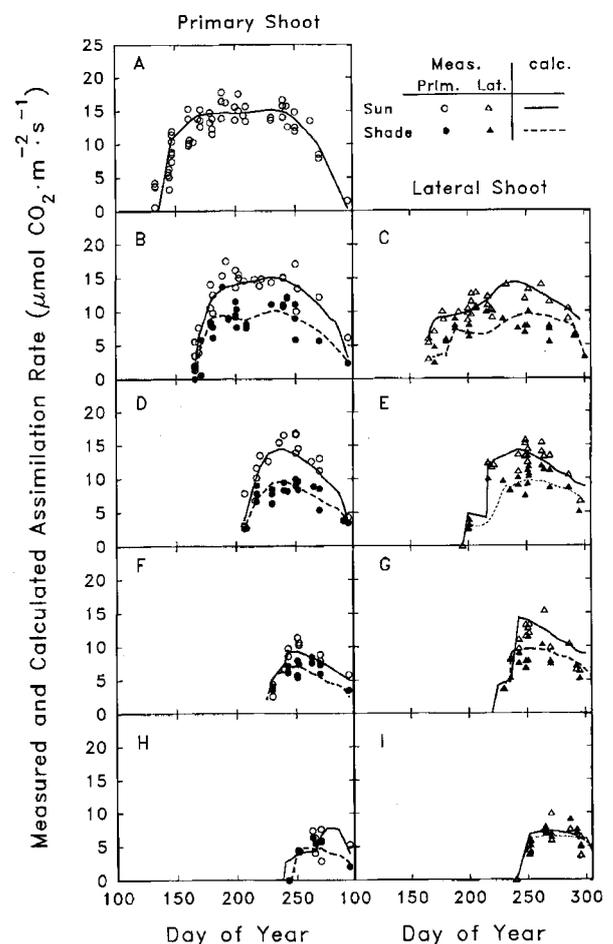


Fig. 2: Photosynthetic rates of sun and shade leaves unfolding at different times during the growing season in relation to their individual physiological age. A, B, D, F, H: leaves on primary shoots, C, E, G, I: leaves on lateral shoots.

above normal ambient CO_2 concentration in 82 % of the leaves measured (61 out of 74 A- c_i curves). Thirteen leaves showed a slight curvilinear response near a c_i of 345 μbar , but the error introduced by estimating stomatal limitation with the resistance analog model was small (see Discussion). Photosynthesis approached CO_2 saturation close to a c_i of 700 μbar for sun, and 550-600 μbar for shade leaves. The slope of the A- c_i curve increased and Γ (intercept at A=0) decreased with increasing LPI for both sun and shade leaves and at all times during the season (Fig. 3 A-D). This indicates an increase in carboxylation efficiency (CE) during leaf ontogeny. During both the pre-veraison and post-veraison phases, the slope of the A- c_i relationship was reduced for shade leaves older than LPI 3 as compared to sun leaves (Fig. 3 A-D). Slopes of apical and basal leaves on lateral shoots were intermediate to those obtained on leaves of primary shoots with LPis 3-6, and LPis 6-10, respectively, pre-veraison, but increased slightly later in the season (data not shown).

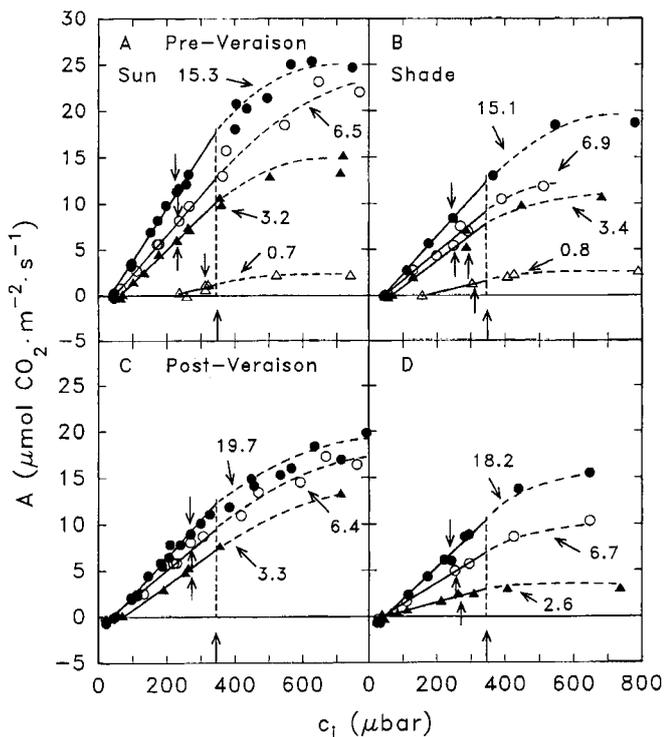


Fig. 3: Response of photosynthesis to c_i for representative sun and shade leaves of different LPis at pre-veraison (A, B), and post-veraison (C, D). Numbers are average LPis of 1-3 leaves used for the A- c_i curves. Continuous lines are linear regressions fitted to data collected between c_a and Γ . Arrows on the abscissa denote $c_a = 345 \mu\text{bar}$. The arrows near the curves indicate A values obtained at $c_a = 345 \mu\text{bar}$. Dashed lines are fitted by eye.

There was no difference in Γ between sun and shade leaves on primary or secondary shoots regardless of age, time of season, and temperature (Fig. 4 A, B; B inset). During the pre-veraison phase, Γ decreased rapidly from about 240 μbar to 50 μbar between LPI 0 and LPI 4 (Fig. 4 A). A minimum Γ of 44 μbar (27.1 $^\circ\text{C}$ leaf temperature) was reached at about LPI 8 (Fig. 4 A). Basal leaves on lateral shoots and fully mature leaves (LPI > 10) on primary shoots had similar compensation points (Fig. 4 A). During the post-veraison phase, an effect of leaf position

on Γ was almost absent for primary shoots and completely absent for lateral shoots (Fig. 4 B). Post-veraison minimum Γ was 41 μbar at 27.1 $^\circ\text{C}$ (Fig. 4 B), declining to about 29 μbar at 20 $^\circ\text{C}$ (Fig. 4 B inset).

Stomatal and non-stomatal limitations of photosynthesis: The increasing c_i during the season (Fig. 1) suggested that the importance of non-stomatal factors in the control of A increased, while stomatal limitation decreased. This was

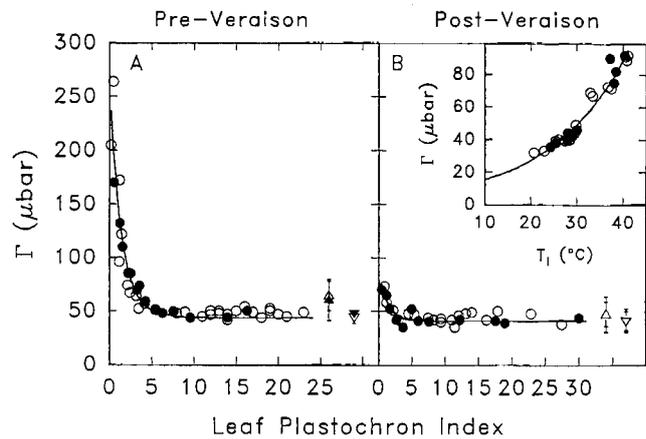


Fig. 4: CO_2 compensation point (Γ) in relation to LPI of sun and shade leaves during the pre-veraison (A), and post-veraison (B) period. Symbols see Fig. 1. Lines represent results of fitting equation 4 to data collected on primary shoots;

$$\text{A: } \Gamma (\mu\text{bar}) = 221.2 \cdot e^{-(0.72 \cdot \text{LPI})} + 43.9, R^2 = 0.97;$$

$$\text{B: } \Gamma = 50.9 \cdot e^{-(0.81 \cdot \text{LPI})} + 41.1, R^2 = 0.99.$$

Values obtained on lateral leaves are presented on the lower right corner of the figures. Each value represents the mean \pm SE of 5-7 leaves. The inset shows the response of Γ of leaves > LPI 10 to leaf temperature measured in the field; $\Gamma = 8.21 \cdot e^{(0.06 \cdot T_l)}$, $R^2 = 0.98$.

confirmed by model calculations of CE and the extent of l to A (Fig. 5). For leaves on primary shoots, l generally decreased from an average (all leaves on a shoot) of 55 % during the early part of the growing season (Fig. 5 G) to about 23 % and 18 % for sun and shade leaves, respectively, at the end of the season (Fig. 5 L), suggesting that non-stomatal factors caused the decline in A. Shade leaf photosynthesis was generally less limited by stomata than sun leaf photosynthesis (Fig. 5 H-L). During the three pre-veraison periods, l decreased with increasing LPis > 6 (Fig. 5 G-I). The post-veraison patterns were less consistent. In contrast, CE was dependent on LPI throughout the season (Fig. 5 A-F). Maximum CE values were generally reached at a LPI of 6-10. The CE declined for older leaves at the end of the season (Fig. 5 E, F). Very early in the growing season, photosynthesis of leaves close to the shoot apex, LPI < 2, was more limited by non-stomatal than stomatal factors (Fig. 5 G), probably related to a very inefficient photosynthetic apparatus. However, when leaves reached LPI 4-5 and CE was approaching maximum, stomata imposed the main limitation to A at this developmental stage (Fig. 5 A, G). The CE of lateral sun and shade leaves increased until the beginning of September (Fig. 5 D), declined thereafter but exceeded the CE of leaves on primary shoots in the late stages of fruit ripening (Fig. 5 E, F).

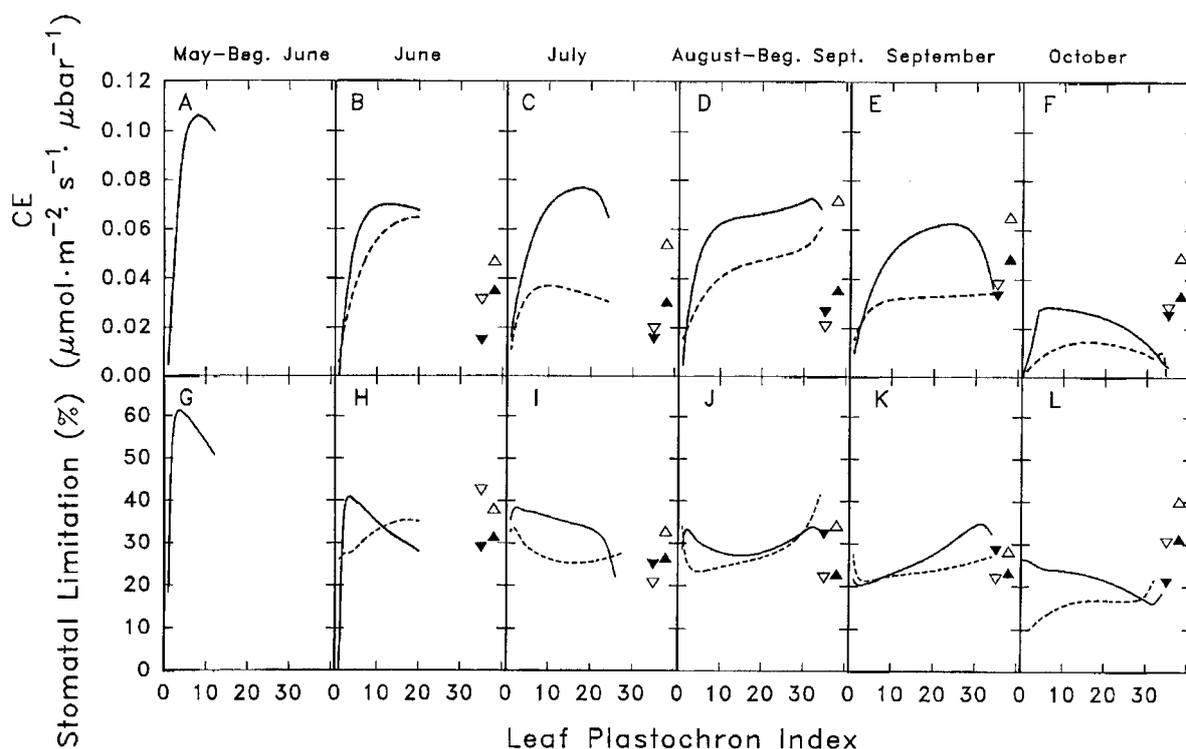


Fig. 5: Estimation of the LPI-dependent carboxylation efficiency (A-F) and stomatal limitation of A (G-L) for sun and shade leaves during 6 periods of the growing season. Continuous and dashed lines, and open and closed triangles, represent calculations for sun and shade leaves on primary and lateral shoots, respectively.

Discussion

The results showed that maximum photosynthesis of sun and shade leaves was similarly related to leaf insertion position as to individual leaf development if leaf age was expressed in plastochrons (Figs. 1, 2). This similarity suggests that one can model the age-dependence of leaf photosynthesis based on their position on the shoot.

A decrease in maximum photosynthetic activity of both sun and shade leaves was not apparent until late in the season, when it occurred simultaneously in all leaves regardless of their physiological age (Fig. 1). By then, some leaves had maintained full photosynthetic activity (near constant A_{max}) for more than 100 days. In contrast, some controlled environment studies on grapevines suggest that peak photosynthesis occurs about 40 days after unfolding with a gradual decline thereafter (KRIEDEMANN 1968; KRIEDEMANN *et al.* 1970; ALLEWELDT *et al.* 1982; INTRIERI *et al.* 1992). These discrepancies may be related to differences in root volume between field and potted plants and the associated differences in hormone levels interacting with stomatal and photosynthetic functioning (BLACKMAN and DAVIES 1984). There are also conflicting results from field studies with grape, some reporting long photosynthetic durations (KOBLET *et al.* 1995), others reporting an early and strong decrease (between pea size and veraison) in A of basal leaves (MOTORINA 1958; HUNTER and VISSER 1988; HUNTER *et al.* 1994; PONI *et al.* 1994 a). The latter type of response seems to be dominant in more arid environments, where premature induction of senescence may have been caused by water deficit or high temperature.

Leaves on lateral shoots maintained their higher photosynthetic activity compared to leaves on primary shoots

during the later stages of the growing season (Figs. 1, 2). In October, carboxylation efficiency for lateral sun leaves was nearly double that of active primary leaves which may be related to a higher nitrogen content per unit leaf area found in lateral leaves until very late in the season (WERMELINGER and KOBLET 1990). Others have stressed the importance of lateral leaves for assimilate supply during the ripening stage of the fruit (KOBLET 1971). In this context, it has also been suggested that the high activity of these leaves might be a response to the sink strength of fruit and wood (KOBLET 1971; SCHOLEFIELD *et al.* 1978), whose demands for carbohydrates may exceed the capacity of the physiologically older primary foliage.

Shading resulted in maximum A and g of about 60-65 % of the sun leaf values (Figs. 1-3). The reduction in A was mainly caused by a decrease in CE (Figs. 3, 5) while Γ remained unaffected, a response commonly observed in shade tolerant species (WALTERS and FIELD 1987). Shade leaves operated at a higher c_i indicating a lower g/CE ratio than sun leaves. Structural and functional modifications in response to low light contributing to decreases in g, A, and CE comprise reductions in stomatal density, Rubisco, protein, and chlorophyll (per unit leaf area) contents, electron transport chains per unit of chlorophyll, and changes in the ratio of photosystem II to photosystem I units (BJÖRKMANN 1982). The reduction in CE indicated a loss of Rubisco activity (VON CAEMMERER and FARQUHAR 1981), which was coupled to a reduction in ribulose-1,5-bisphosphate (RuBP) regeneration capacity, as indicated by the lower CO_2 saturated photosynthetic rates of shade leaves (Fig. 3) (WALTERS and FIELD 1987). Both effects increased with increasing LPI. A strong reduction in carboxylation capacity triggered by low light has also been

shown for other woody species (LANGENHEIM *et al.* 1984), while a recent study by IACONO *et al.* (1995) on grapevines reported only a reduction in stomatal conductance but not in CO₂ assimilation and CE after shading whole vines at veraison.

The linear response of both sun and shade leaves to an increase in internal CO₂ partial pressure of up to 345 µbar indicated that both leaf types operated in the RuBP saturated part of the A-c_i curve and that at all leaf ages carboxylation capacity was limiting photosynthesis more than electron transport rates and RuBP regeneration. Maximum photosynthetic activity in shade leaves was reached at a LPI between 6 and 7, 3-4 plastochrons earlier than in sun leaves and coinciding with the completion of leaf lamina expansion in grape. Similar to sun leaves, shade leaves maintained a high photosynthetic capacity for over 100 days.

The c_i was similar between leaves of different ages over most of the season with the exception of the early part of the season. Apparently stomatal function and structure developed in an asynchronous manner to the photosynthetic apparatus and chlorophyll content (KRIEDEMANN 1968), causing CE and c_i to vary with age (DÜRING 1994).

The general increase in c_i across all leaves as the season progressed, which was more pronounced in shade leaves, suggested a decline in water use efficiency, WUE, and stomatal limitation to A (SCHULZE and HALL 1982). This type of response to time of the season has been reported for several plant species (SOLÁROVÁ and POSPÍŠILOVÁ 1983), but was absent in a study on grape under more arid conditions (PONI *et al.* 1994 a). It is difficult in this context to separate the effects of the environment, especially humidity, from the effects of age on g and A.

Estimates of l from field data were based on the linear resistance analog model (JONES 1985). It requires that the A-c_i relationship is linear between Γ and c_i values equivalent to normal ambient CO₂ partial pressures. In grape, 82 % of the A-c_i curves conducted (61 of 74) were linear up to 345 µbar, in 18 % of the curves was the break point at c_i values lower than 345 µbar. Using the resistance analog to calculate l for the latter cases yielded a 5.2 % (SE ± 1.6) overestimation. For the entire data set of 74 A-c_i curves, this resulted in an average error of less than 1 %. Strong linearity in A-c_i relationships for grape leaves up to c_i values larger than the ambient CO₂ partial pressures have been reported for field (CHAVES *et al.* 1987) and laboratory studies but seems to depend on the time of day when measurements are conducted (DÜRING 1991).

The interpretation of c_i data is difficult when environmental stresses (water stress, salinity, low humidity) are present due to the non-uniform aperture of stomata over the surface of a leaf (patchiness) (DOWNTON *et al.* 1990; DÜRING and LOVEYS 1996). Recent findings suggest that even sudden changes in light intensity may cause patchiness (DÜRING and LOVEYS 1996), yet low humidity may be necessary for this to occur (ECKSTEIN *et al.* 1996). The occurrence of stomatal patches in our experiments with exposed shade leaves remains a possibility, yet seems unlikely since plants were always well-watered, humidity was high and stomatal conductance maximal.

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Appendix: Estimated parameter values of the non-linear regression analyses of equations 1 and 2, for the relationships of A and g_H to LPI for sun and shade leaves of primary shoots in different periods during the growing season. Average A_{max} and g_{max} values are also given for basal and apical lateral leaves. Phenological phases I-III are pre-veraison; and IV-VI post-veraison.

Primary shoot								Lateral shoot	
Variable	Period	$A(g_H)$ max	α	β	γ	LPI _{max}	R ²	basal	apical
								$A(g_H)$ max	$A(g_H)$ max
A sun	I	15.3	0.34	0.83			0.96		
	II	14.7	0.28	0.95			0.98	8.82	5.10
	III	15.0	0.24	0.54			0.98	11.00	4.45
	IV	14.6	0.30	0.82	1.21	36.47	0.97	14.15	4.51
	V	14.2	0.19	0.10	0.22	37.49	0.97	14.07	8.79
	VI	6.9	1.21	1.86	0.11	36.09	0.94	8.79	5.82
A shade	II	12.8	0.23	0.73			0.97	7.31	3.00
	III	8.5	0.39	1.03			0.98	6.74	3.31
	IV	10.8	0.19	-0.62			0.96	8.18	4.92
	V	7.6	0.39	-0.35			0.93	11.16	7.08
	VI	5.4	0.14	0.04	0.06	39.67	0.88	6.86	5.98
	g_H sun	I	195.9	0.15	-0.52			0.96	
II		371.4	0.10	-0.06			0.98	117.7	54.7
III		305.8	0.12	-0.46			0.98	189.1	109.2
IV		481.6	0.15	0.69	0.03	53.52	0.98	234.3	82.4
V		397.1	0.17	-0.99	0.04	47.04	0.94	286.8	227.2
VI		154.2	1.07	1.70	0.63	35.27	0.95	120.4	67.0
g_H shade	II	208.8	0.35	0.64			0.96	142.9	58.9
	III	424.4	0.17	0.06	0.02	43.78	0.96	143.9	75.3
	IV	250.1	0.23	0.06	0.14	40.11	0.97	188.5	78.6
	V	200.2	0.49	0.15	0.07	57.41	0.94	276.4	137.7
	VI	129.3	0.30	-0.28	0.16	37.01	0.99	120.5	63.4