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Determination of the photosynthetic capacity of grapevine leaves

by

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Bestimmung der Photosynthesekapazität in Rebblättern

Zusammenfassung: An Blättern ausreichend bewässerter Topfreben im Freiland wurden vormittags und nachmittags Messungen der stomatären Leitfähigkeit und der Photosynthese durchgeführt, wobei nachmittags stets eine geringere Photosynthesekapazität (maximale Photosyntheserate bei Licht- und CO_2-Sättigung) ermittelt wurde. Dieser Befund war mit einer höheren Empfindlichkeit der Stomata gegenüber CO_2 am Nachmittag verbunden: 0,0016 μbar^{-1} am Vormittag, 0,0046 μbar^{-1} am Nachmittag. Eine Bestimmung der Photosynthesekapazität am Morgen bei 1—2 % O_2 (Hemmung der Lichtatmung) ergab maximale Photosynthesewerte bereits bei geringeren CO_2-Partialdrücken. Dies führte dazu, daß die Photosynthesekapazität bei maximaler stomatärer Leitfähigkeit bestimmt werden konnte. Wurde von den so ermittelten Photosynthesekapazitätswerten der Anteil der Lichtatmung in Abzug gebracht, erreichte die Photosynthesekapazität Werte, die denen bei 21 % O_2 und höheren CO_2-Partialdrücken entsprachen. Dies läßt den Schluß zu, daß in den vorliegenden Untersuchungen am Morgen die Photosyntheserate durch Stomata nicht vermindert war. Zur Verhinderung von Photosyntheseeinbußen infolge Lichthemmung wird vorgeschlagen, die Dauer des Einsatzes von Starklicht zu minimieren und die Lichtintensität auf Werte zu reduzieren, bei denen eine Lichtsättigung gerade erreicht ist.

Key words: leaf, stoma, light, photosynthesis, photosynthetic capacity, carboxylation efficiency, stomatal conductance, respiration, photorespiration, method.

Introduction

In numerous experiments the photosynthetic rate of leaves of grapevines has been determined at optimum humidity and temperature conditions and light saturation, e.g. to compare varieties or to study effects of manipulations (e.g. Kriedemann and Lenz 1972; Chaves *et al.* 1987; Düring 1988; Roper and Williams 1989).

This 'maximum photosynthetic activity' of Riesling leaves varied over a large scale (3.2—13.0 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) among several publications (Schultz 1989). At least in part this variation is due to stomatal conductance, which in grapevine leaves is closely correlated to the rate of photosynthetic CO_2 assimilation and which fully accounts for the inhibition of CO_2 assimilation under conditions of moderate water stress by limiting the CO_2 supply to the mesophyll (Downton *et al.* 1988 b; Düring 1987, 1988, 1990). In order to overcome stomatal limitation of CO_2 supply Lange *et al.* (1986) determined the 'photosynthetic capacity', i.e. the maximal net photosynthetic CO_2 assimilation under saturating light conditions and at saturating CO_2 partial pressure. Saturation of photosynthetic carboxylation reactions with CO_2 appears to be a promising method to eliminate stomatal influences. However, increasing CO_2 partial pressures may have a stomata closing effect, which in grapevine leaves has been shown to occur in a non-uniform manner (Raschke 1975; Downton *et al.* 1988 a, b). The net effect of this non-uniform stomatal behaviour (some groups of stomata close while others remain open) is a reduction in functional leaf area which can lead to erroneous determinations of the photosynthetic capacity. In addition, in glasshouse-grown vines non-stomatal limitations of photosynthetic capacity are reported after prolonged high light conditions (CORREIA *et al.* 1990).

The aim of this paper is to examine the responses of stomatal conductance and CO_2 assimilation to increasing ambient CO_2 partial pressures during different periods of the day and to propose experimental conditions where the determination of the photosynthetic capacity is not disturbed by stomatal or non-stomatal interference.

Material and methods

3-year-old container-grown Riesling vines grafted to rootstock Kober 5 BB were cultivated outside the glasshouse. Plants were fully irrigated in the evening to keep the leaf water potential above −8 bar during the day. Gas exchange measurements were performed using a Miniküvetten-System (H. Walz, D-8521 Effeltrich, FRG) which has been described earlier (LANGE *et al.* 1986; DÜRING 1988). At the end of June 1990 the responses of CO_2 assimilation (A) and stomatal conductance (g) to various ambient CO_2 partial pressures were determined in the morning from 9 to 11 a.m. and in the afternoon from 1 to 3 p.m.. Parts of fully expanded but not senescent leaves in the middle part of the vertically trained canes (length: 1.80 m) were inserted into the gas exchange chamber. The cuvette temperature was kept constant at 22 °C, incoming air humidity was controlled at a dew point of 14 °C, thereby producing a relative humidity of about 65 %. Saturating light (1180 μmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$) was provided for the enclosed part of the leaf with a 12 V/75 W projector lamp and gray filters. CO_2 partial pressure was controlled by a mass-flow-metered gas mixing system and an infrared gas analyser (Binos 1.4 B, Leybold-Heraeus, Hanau, FRG) in the range of 50—2000 μbar. Data obtained for differentials in CO_2 and water vapour, light, temperature and air humidity were stored by a data acquisition system (DES, H. Walz) and computed according to VON CAEMMERER and FARQUHAR (1981). Photorespiration was calculated according to SHARKEY (1988). Stomatal sensitivity to CO_2 at 350 μbar was determined as $\Delta g/g \cdot \Delta c_i$.

A, g, c_i (intercellular CO_2 partial pressure) and CE (carboxylation efficiency) values which were calculated from data obtained in the afternoon under the assumption of homogeneous stomatal aperture on the leaf surface will be marked with an asterisk (*) in the following text.

Results and discussion

Fig. 1 shows the responses of stomatal conductance for CO_2 (g_{CO_2}) and CO_2 assimilation (A) to changes in ambient CO_2 partial pressure (c_a) under conditions of saturating light intensity and constant air humidity and temperature. Measurements in the morning indicate an increase of g_{CO_2} up to a maximum at c_a = 450 μbar and a subsequent decrease to about 40 % of the maximum at c_a = 2000 μbar. The photosynthetic activity plotted against c_a obviously results in a saturation-type curve, the initial slope, representing the carboxylation efficiency, being almost linear. CO_2 saturation of the CO_2 fixation sites in the chloroplasts is reached at about c_a = 1400 μbar which corresponds to a calculated intercellular CO_2 partial pressure of about 1180 μbar and a CO_2 assimilation rate of 16.4 μmol $\cdot$ $m^{-2} \cdot s^{-1}$. At higher c_a values the rate of CO_2 assimilation slightly declines. A similar decline of the photosynthetic activity was observed by STOEV and SLAVTCHEVA (1982). The leaf was then maintained in the cuvette at 350 μbar CO_2 and at saturating light conditions for 2 h.

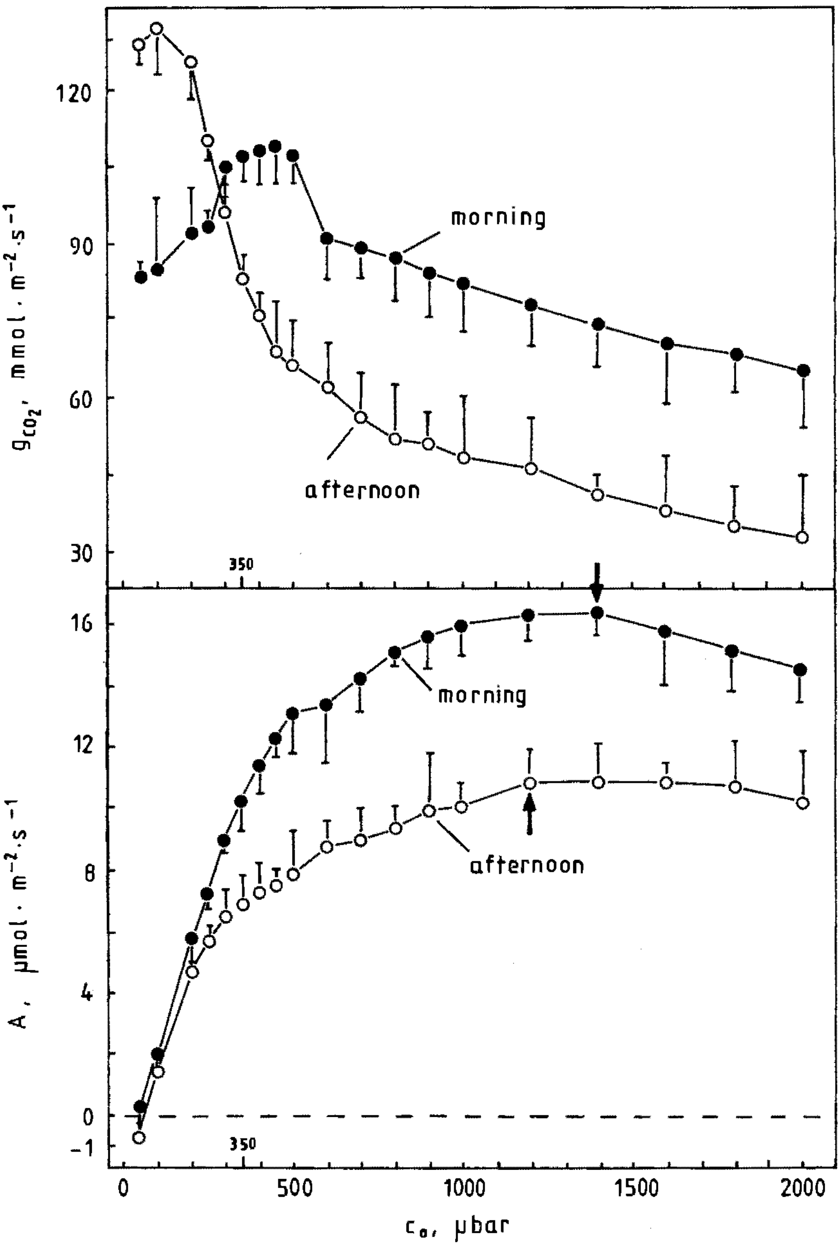

Fig. 1: Stomatal conductance (g_{CO_2}*) and photosynthesis (A*) of leaves of Riesling vines related to ambient CO_2 partial pressure (c_a). Measurements were taken in the morning (9—11 a.m.) and the afternoon (1—3 p.m.). Arrows indicate photosynthetic capacity. The vertical bars indicate least significant differences at the 5 % level.

Die stomatäre Leitfähigkeit (g_{CO_2}*) und Photosynthese (A*) von Blättern der Sorte Riesling in Abhängigkeit vom CO_2-Partialdruck der Außenluft (c_a). Die Messungen wurden vormittags (9—11 Uhr) und nachmittags (13—15 Uhr) durchgeführt. Die Pfeile verweisen auf die Photosynthesekapazität. Senkrechte Balken: Grenzdifferenzen bei 5 %.

A repetition of the experiment in the afternoon at the same climatic conditions shows that stomatal conductance* and CO_2 assimilation* responses to increasing CO_2 partial pressures were different from those obtained in the morning. At low c_a values stomatal conductance* is high (about 130 mmol · m^{-2} · s^{-1}) but declines steadily when c_a surpasses 100 µbar to values which are about 75 % beyond the initial maximum. At

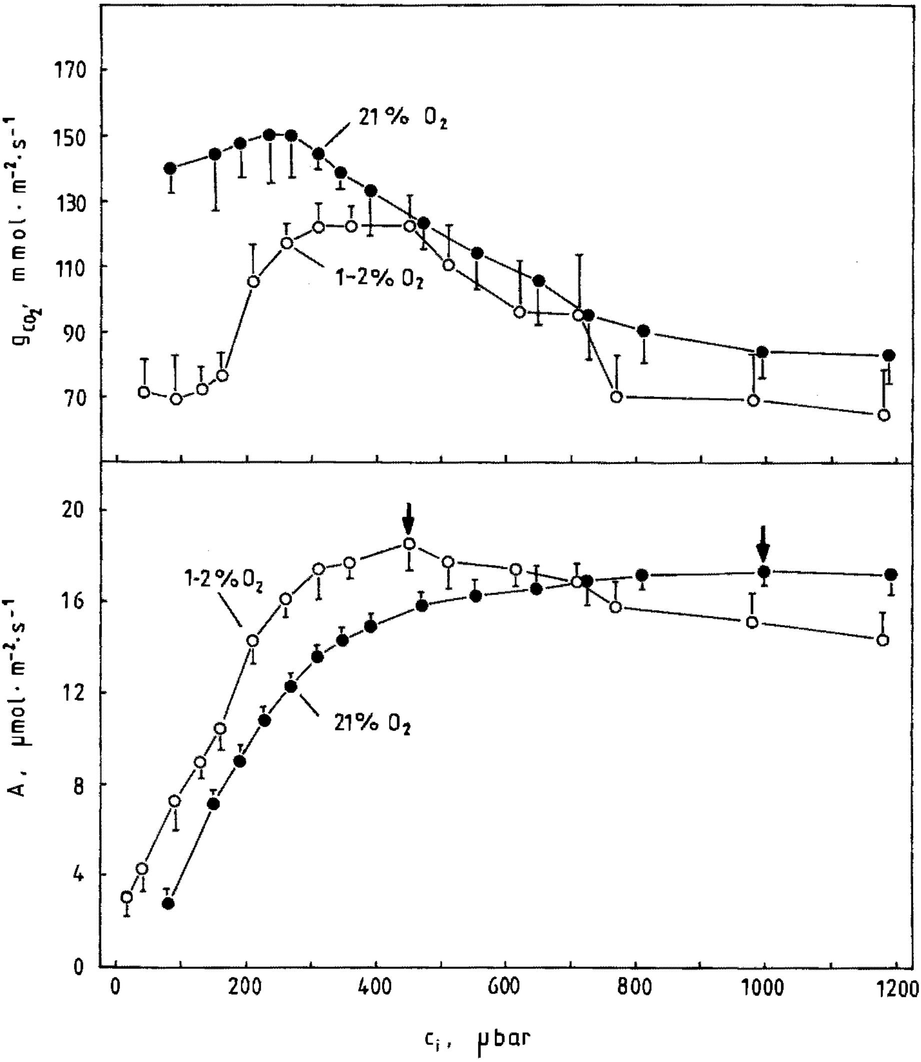

Fig. 2: Stomatal conductance (g_{CO_2}) and photosynthesis (A) of leaves of Riesling vines related to intercellular CO_2 partial pressure (c_i). Measurements were taken in the morning (9—11 a.m.) at 21% O_2 or 1—2 % O_2. Arrows indicate photosynthetic capacity. The vertical bars indicate least significant differences at the 5 % level.

Die stomatäre Leitfähigkeit (g_{CO_2}) und Photosynthese (A) von Blättern der Sorte Riesling in Abhängigkeit vom interzellulären CO_2-Partialdruck (c_i). Die Messungen wurden vormittags (9—11 Uhr) bei 21 % O_2 oder 1—2 % O_2 durchgeführt. Die Pfeile verweisen auf die Photosynthesekapazität. Senkrechte Balken: Grenzdifferenzen bei 5 %.

CO_2 partial pressures ≥ 200 µbar photosynthesis* increases, but to a distinctly lower extent than in the morning. The maximum rate of photosynthesis* (10.8 µmol · m^{-2} · s^{-1}) is reached at c_a = 1200 µbar, 2000 µbar CO_2 partial pressure causing a small reduction of CO_2 assimilation*. It is interesting to note that at low c_a values (200—250 µbar) the rates of CO_2 assimilation* in the afternoon are somewhat lower than the corresponding values in the morning, although stomatal conductance* at 100 to 250 µbar CO_2 is higher in the afternoon. As a consequence, calculated intercellular CO_2 partial pressure* is higher and the carboxylation efficiency* is reduced in the afternoon. This may be a hint at mesophyll limitations of photosynthesis.

In another series of experiments (data not shown) stomatal conductance and the photosynthetic activity were also reduced in the morning when c_a values surpassed 350—450 µbar, while in the afternoon this decrease already started at c_a ≥ 100—250 µbar. Calculation of g* sensitivity to CO_2 at 350 µbar CO_2 partial pressure indicates higher CO_2 sensitivity in the afternoon (0.0046 $µbar^{-1}$) compared to the morning (0.0016 $µbar^{-1}$); this tendency agrees with results of CORREIA *et al.* (1990).

Stomata of *Vitis vinifera* are reported to be particularly sensitive to CO_2 (LOVEYS and DÜRING 1984). RASCHKE (1975) has pointed out that stomata can be sensitized to CO_2 by raising the abscisic acid (ABA) level in leaves. Our earlier results have shown that ABA increased in vine leaves in the morning and reached a peak of approximately twice the pre-dawn level at midday (LOVEYS and DÜRING 1984; LOVEYS 1984). Thus, the increased sensitization of stomata to CO_2 in the afternoon may have been mediated by ABA.

It may be argued that even in the morning the determination of photosynthetic capacity may be incorrect, due to stomatal limitation at high CO_2 partial pressures. As has been shown earlier under normal atmospheric conditions, photosynthesis of vine leaves is subjected to considerable oxygen inhibition, photorespiration accounting for approximately 20 % of photosynthesis in non-stressed plants (DÜRING 1988). Determination of photosynthesis at low O_2 conditions (1—2 %) will reduce photorespiration to zero without affecting stomatal conductance (KRIEDEMANN and DOWNTON 1981; DOWNTON *et al.* 1987). In an experiment carried out in the morning with 1—2 % O_2, the carboxylation efficiency is improved by about 40 % compared to measurements at 21 % O_2, as calculated from the data of Fig. 2. The maximum rate of A (18.5 µmol · m^{-2} · s^{-1}) is already reached at c_i = 450 µbar (or c_a = 600 µbar), i.e. at a distinctly lower CO_2 partial pressure compared to the measurement at 21 % O_2 (Fig. 2). At c_i = 450 µbar stomatal conductance is still at its maximum (122 mmol · m^{-2} · s^{-1}), only further increases of CO_2 lead to slight decreases of g and A. The maximum value of A can be corrected for photorespiration. Fig. 3 demonstrates that rates of photorespiration *per se* or related to A decrease at increasing c_i. At c_i = 450 µbar (A maximum) photorespiration is diminished to about 10 % of A. As the calculated A capacity (18.5 − 10 % = 16.7 µmol · m^{-2} · s^{-1}) and the A capacity determined at the same leaf at ambient O_2 conditions (17.2 µmol · m^{-2} · s^{-1}) are quite close it is suggested that the distinct decline of stomatal conductance from 150 to 84 mmol · m^{-2} · s^{-1} (Fig. 2) obviously had no limiting effect on photosynthesis.

Conclusion

A decline of carboxylation efficiency and photosynthetic capacity at noon or in the afternoon has already been reported for *Arbutus unedo* (BEYSCHLAG 1984; RASCHKE und RESEMANN 1986; DEMMIG-ADAMS *et al.* 1989) and *Quercus suber* (TENHUNEN *et al.* 1984). Several hypotheses have been established to explain the phenomenon, including stomatal closure due to decreases of plant water potential or air humidity (SCHULZE 1986), feed-back inhibition due to the accumulation of carbohydrates (AZCON-BIETO 1986) and inhibitory effects after prolonged high light exposure of leaves due to photoinhibition (CORREIA *et al.* 1990).

The present results indicate that the reductions of carboxylation efficiency and photosynthetic capacity which had been demonstrated for leaves of glasshouse-grown vines (CORREIA *et al.* 1990) also occur under outdoor conditions. Although the causes of limited photosynthesis in the afternoon are still rather obscure, it appears that stomatal and non-stomatal effects are involved. There is strong evidence now that, (1) determination of the photosynthetic capacity of grapevines should be restricted to the morning when stomatal sensitivity to CO_2 obviously is low and (2) prolonged exposure of leaves to high light intensities should be avoided to attain reproducible data of photosynthetic capacity.

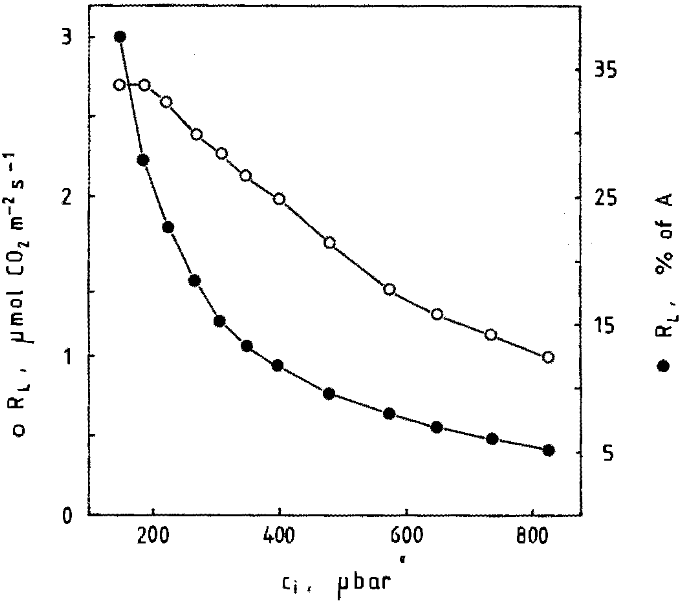

Fig. 3: Photorespiration (R_L) of leaves of Riesling vines related to the intercellular CO_2 partial pressure (c_i). Measurements were taken in the morning (9—11 a.m.) at 21 % O_2.

Die Lichtatmung (R_L) von Blättern der Sorte Riesling in Abhängigkeit vom interzellulären CO_2-Partialdruck (c_i). Die Messungen wurden vormittags (9—11 Uhr) bei 21 % O_2 durchgeführt.

Summary

At fully irrigated, container-grown vines (cv. Riesling) kept outdoor, measurements of stomatal conductance and photosynthesis of leaves were performed in the morning and in the afternoon. The results revealed reductions of the photosynthetic capacity (maximal net photosynthesis at saturating light conditions and at saturating CO_2 partial pressure) in the afternoon. This observation was associated with a higher sensitivity of stomata to CO_2 in the afternoon: 0.0016 μbar^{-1} in the morning, 0.0046 μbar^{-1} in the afternoon. Due to inhibition of photorespiration determinations of

photosynthetic capacity in the morning at 1—2 % O_2 revealed maximal values at lower CO_2 partial pressures and, thereby, at maximal stomatal conductance. These values, corrected for photorespiration, were close to those obtained at ambient O_2 and high CO_2 partial pressures. Thus, it is assumed that in our experiments stomata did not limit the rates of photosynthesis which were determined in the morning. To avoid reduction of photosynthesis by photoinhibition it is proposed to minimize the duration of exposure of leaves to high light and to reduce light intensity to values where photosynthesis begins to reach light saturation.

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