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Stomatal and mesophyll conductances control CO_2 transfer to chloroplasts in leaves of grapevine (*Vitis vinifera* L.)

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Summary

From simultaneous determination of net CO_2 assimilation and transpiration at the abaxial side and of the photosynthetic electron transport rate at the adaxial side of field-grown, light-saturated leaves of grapevine (cv. Riesling) photorespiration, stomatal conductance for CO_2, mesophyll conductance and the CO_2 concentration in intercellular spaces (Ci) and in chloroplasts (Cc) were estimated. CO_2 assimilation was saturated at about Ci = 340 ppm. At increasing ambient CO_2 concentration (Ca) photorespiration decreased (less negative values); stomatal conductance decreased significantly (- 45 %) limiting CO_2 uptake into intercellular spaces. Rates of total photosynthetic electron transport were constant between Ci = 340 and 800 ppm and decreased by 34 % at low Ci. Electron flow to carboxylation was closely correlated to CO_2 assimilation rates (R^2 = 0.999). When Ca was raised, the CO_2 concentration in chloroplasts (Cc) increased but at smaller rates than Ci. Presumably due to the distinct decline of the mesophyll conductance Cc remained constant at Ci >340 ppm. At Ca = 400 ppm the Cc/Ca ratio was 0.46 - 0.48, corroborating data reported for other species (Cornic and Fresneau 2002). At 2 % ambient O_2 and 400 ppm CO_2 decreased rates of photorespiration (- 69 %) were associated with a decline of total photosynthetic electron flow (- 6 %); higher stomatal and mesophyll conductances, however, led to increases of Cc and CO_2 assimilation rates (+ 49 %). It is hypothesized that both stomatal and mesophyll conductance are involved in the adaptation of the CO_2 supply to the CO_2 demand at the site of carboxylation in chloroplasts.

K e y w o r d s : photosynthesis, photorespiration, photosynthetic electron transport, chloroplastic carbon dioxide, stomatal conductance, mesophyll conductance.

Introduction

Leaves of grapevines are characterised by relative low rates of CO_2 assimilation (generally $\leq$ 20 µmol CO_2 m^{-2} s^{-1}) under saturating light, ambient CO_2 concentration and favourable air humidity and temperature condition as compared to some herbaceous plants with values up to 40 µmol CO_2 m^{-2} s^{-1} (Larcher 1975, Düring 1991, Epron *et al.* 1995).

This characteristic of woody plants may be associated with biochemical constraints on CO_2 assimilation, *i.e.* Rubisco activity or chloroplast capacity for electron transport and/or limitations of the CO_2 transfer rate from the ambient air to the chloroplasts (Lloyd *et al.* 1992).

Stomatal conductance for CO_2 (g_{CO2}) of grape leaves generally varies between 0 and 200 mmol m^{-2} s^{-1} and, at ambient CO_2 concentration (350-380 ppm), intercellular CO_2 concentration (Ci) ranges from 250 to 300 ppm. These values are not different from other C_3 plants (Lloyd *et al.* 1992). According to Bota *et al.* (2002) the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity in leaves of field-grown grapevine (cv. Tempranillo) was close to the light- and CO_2-saturation rate of photosynthesis and in our experiments with field-grown grape varieties total electron transport rates (J_t) ranged from 80 to 190 µmol e^- m^{-2} s^{-1} (Ortoidze and Düring 2001). Since these values are well above those required for CO_2 assimilation (Lloyd *et al.* 1992), it has been suggested that CO_2 assimilation in woody plants may be limited by low internal ('mesophyll') conductance for CO_2 (g_{mes}), *i.e.* the diffusion of CO_2 through mesophyll cell walls, the plasmalemma, part of the cytosol, and the chloroplast envelope and some of the chloroplast stroma (Nobel 1983, Epron *et al.* 1995, Lawlor 2002).

Lawlor and Cornic (2002) assumed that under optimum ambient conditions in fully hydrated leaves CO_2 assimilation can be increased to the potential rate of photosynthesis (A_{pot}) by increasing Ca to 800 ppm, on the premise that "g_m (equals g_{mes}, the author) is not changed when Ca increases"; thus, at increasing Ca and Ci changes of the CO_2 transfer in the mesophyll can not be excluded *a priori*.

The aim of the present study was to elucidate the CO_2 transfer in leaves of irrigated grapevines by determining stomatal and mesophyll conductances as well as intercellular and chloroplastic CO_2 concentrations as a function of increasing ambient CO_2 concentration.

Material and Methods

In June 2002 the distal end of shoots from field-grown Riesling vines with 6-8 fully expanded leaves were cut and immediately re-cut under water to avoid air embolism in xylem vessels. During and after transfer to the laboratory the shoots were kept in water. Light was provided by two lamps (Powerstar HQI–T, 400 W/DH, Osram). The leaf water potential under these conditions ranged between -0.25 and -0.35 Mpa.

C o m b i n e d g a s e x c h a n g e a n d c h l o r o p h y l l f l u o r e s c e n c e m e a s u r e m e n t s : Fully expanded leaves were used to measure simultaneously gas

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exchange and chlorophyll fluorescence at the same leaf segment using a 'HCM-1000-Photosynthesis-System' (Walz, Effeltrich, Germany) combined with a 'Mini-PAM-System' (Walz, Germany).

Leaves were inserted into the cuvette to measure gas exchange at their abaxial side and simultaneously chlorophyll fluorescence (quantum yield) at the adaxial side.

Measurements were performed at light-saturation of CO_2 assimilation (750 µmol m^{-2} s^{-1}) between 8 and 11 a.m. Leaf temperature was kept constant at 25 °C, relative air humidity in the cuvette at 50-55 %. CO_2 concentration of the gas stream leading to the measuring cuvette with the inserted leaf was increased stepwise from 50 to 1000 or 2000 ppm CO_2, respectively. The net CO_2 assimilation rate (A), the stomatal conductance for CO_2 (g_{CO2}) and the intercellular CO_2 concentration (Ci) were calculated according to VON CAEMMERER and FARQUHAR (1981) and the quantum yield of the illuminated leaf area (Fv/Fm') was determined by saturating light pulses as described earlier (DÜRING 1998, DÜRING and DAVTYAN 2001).

According to KRALL and EDWARDS (1992) the total electron flow (J_t) can be derived from the quantum yield of PSII (Y), the light intensity incident on the leaf (PAR), the fractional absorptance of light by the leaf (a) and the absorptance by PSII divided by the absorptance of PSI + PSII (f):

$$J_t = Y \cdot PAR \cdot a \cdot f,$$

where 'a' equals 0.84 and 'f' equals 0.5 (SCHREIBER 1997).

J_t can be divided into its components, J_c and J_o, the electron flow rates devoted to carboxylation and oxygenation of RuBP according to EPRON *et al.* (1995):

$$J_c = 1/3\,[J_t + 8(A + R_d)] \text{ and } J_o = 2/3\,[J_t - 4(A + R_d)],$$

where A is the CO_2 assimilation rate and R_d the dark respiration. R_d was determined by gas exchange measurements after keeping leaves in the dark for 10 min.

Photorespiration (R_L) was calculated according to VALENTINI *et al.* (1995):

$$R_L = 1/12\,[J_t - 4\,(A + R_d)]$$

The CO_2 concentration at the chloroplast level, Cc, was determined according to CORNIC and FRESNEAU (2002) assuming a specificity factor of 80, which is commonly used for C_3 plants:

$$Cc = 2\Gamma\,(J_c / J_o),$$

where $\Gamma = 42.5$ ppm at a leaf temperature of 25 °C. Meanwhile BOTA *et al.* (2002) have reported somewhat lower specificity factors for leaves of grapevine, the value depending, *inter alia,* on variety and leaf age.

The mesophyll (or 'internal') conductance (g_{mes}) was calculated as proposed by HARLEY *et al.* (1992):

$$g_{mes} = A / Ci - Cc$$

Results presented are mean values of 4-5 replicates, bars denote confidence limits at the 5 % level.

Results and Discussion

Under saturating light conditions CO_2 assimilation rates (A) of Riesling leaves increased with increasing intercellular CO_2 concentration (Ci) induced by stepwise increases of ambient CO_2 concentration (Ca) (Fig. 1). Photosynthesis was almost CO_2-saturated at Ci = 340 and increased only slightly

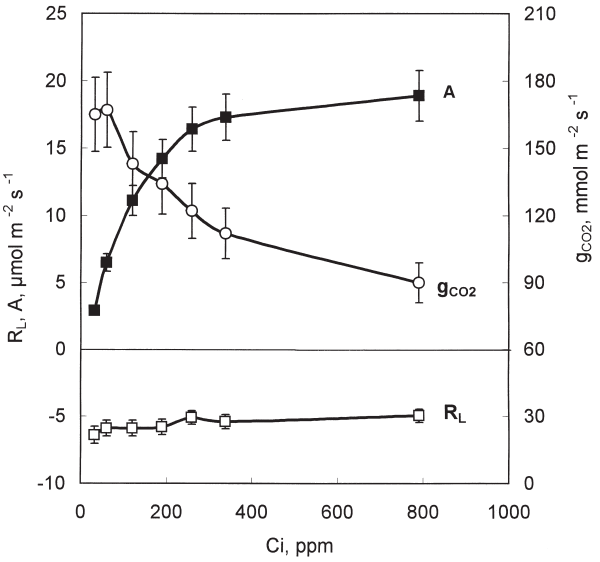

Fig. 1 CO_2 assimilation (A), stomatal conductance for CO_2 (g_{CO2}) and photorespiration (R_L) as a function of intercellular CO_2 concentration (Ci). Bars denote confidence limits at the 5 % level.

at higher Ci. Concurrently photorespiration (R_L) decreased by 29 % (values less negative) and stomatal conductance (g_{CO2}) by 45 %.

It has been shown earlier that stomatal pore size of grapevine leaves declines at increasing Ca, the sensitivity of stomata to CO_2 being higher in dehydrated leaves (DÜRING 1991). According to RASCHKE (1975) the stomatal response to CO_2 can be ascribed to increases of CO_2 in the substomatal cavity and the pore, *i.e.* to Ci rather than to Ca.

The total electron transport rate (J_t) and its component devoted to carboxylation, J_c, were constant at high Ci (340 and 800 ppm) and decreased by 34 % at low Ci (Fig. 2); J_c was closely related to rates of CO_2 assimilation (Fig. 3). Interestingly, at low Ci (32 ppm) A was close to zero while J_t decreased only by 34 %.

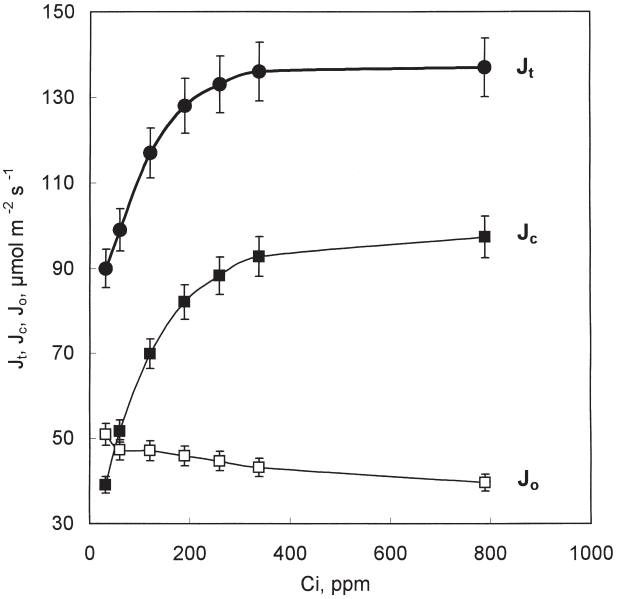

Fig. 2 Total electron flow (J_t), electron flow to carboxylation (J_c) and to oxygenation (J_o) as a function of the intercellular CO_2 concentration (Ci). For details see Fig. 1.

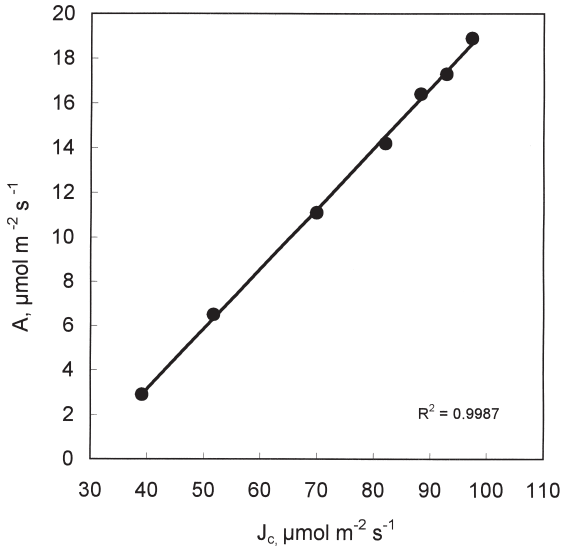

Fig. 3 The relationship between CO_2 assimilation (A) and the electron flow to carboxylation (J_c).

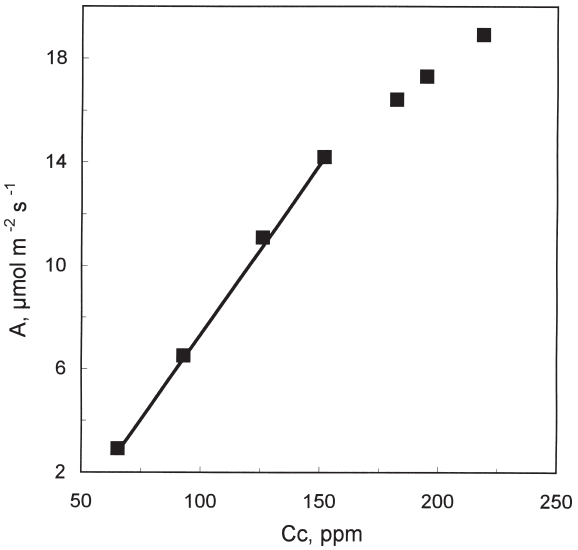

Fig. 4 The relationship between CO_2 assimilation (A) and the chloroplastic CO_2 concentration (Cc).

Similar results have been reported by Cornic and Briantais (1991) and Cornic and Fresneau (2002) who concluded that the photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during a mild drought, *i.e.* when CO_2 supply to chloroplasts is limited. Obviously, under these conditions oxygen can efficiently replace carbon dioxide as an electron acceptor. In their experiments at low Ci or in dehydrated leaves the allocation of photosynthetic electrons to O_2 was increased. This corroborates our data indicating that at Ci = 32 ppm electron flow to O_2 reduction (J_o) was higher than the electron flow to CO_2 reduction (J_c) (Fig. 2).

The relationship between A and Cc is shown in Fig. 4. The initial linear slope of the curve represents the carboxylation efficiency, *i.e.* the stage where RuBP is saturated (Farqhuar and Sharkey 1982). In the past the carboxylation efficiency was approximately derived from A/Ci relationships, however, under various stress conditions estimation of Ci in leaves of grapevine (and some other 'heterobaric' species) is misleading due to the heterogenous behaviour of stomata ('stomatal patchiness') (review: Weyers and Lawson 1997, grapevine: Düring and Loveys 1996). Relating A to Cc instead of Ci appears to be a most promising way to by-pass the problem and to determine carboxylation efficiency at the site of carboxylation, *i.e*. more accurately.

The almost linear increase of Ci with Ca (Fig. 5) demonstrates that CO_2 uptake into substomatal cavities and intercellular spaces was limited only to a small extent by stomata, *e.g.* at Ca = 1000 ppm Ci was reduced only by 21 %.

Like Ci, Cc increased when Ca was raised, however, at somewhat smaller rates; *e.g.*, at Ca = 400 ppm Cc was lower than Ci (-77 ppm or -30 %). This is in line with observations of Krall and Edwards (1992) who assumed a 10 % decrease in CO_2 in the chloroplasts relative to the concentration in the substomatal cavity "due to diffusive resistance in the aqueous phase" and considerably higher values for leaves of woody plants. Similar results were obtained from 13 woody plants by Epron *et al*. (1995) who concluded that "the low values of net CO_2 uptake that occur in some woody plants

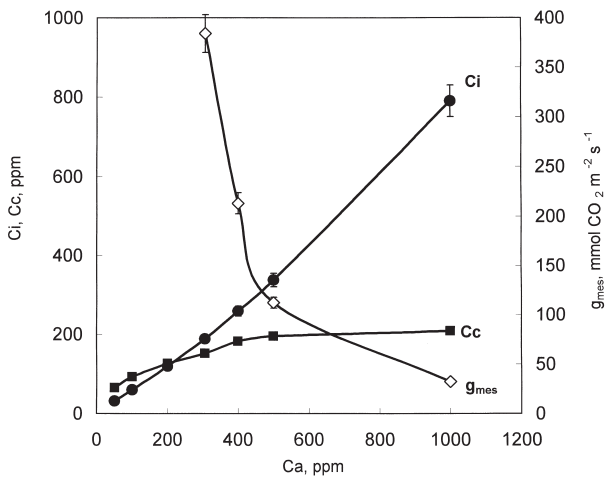

Fig. 5 The intercellular CO_2 concentration (Ci), the chloroplastic CO_2 concentration (Cc) and the mesophyll conductance (g_{mes}) as a function of ambient CO_2 concentration (Ca). For details see Fig. 1.

are partly ascribable to high internal resistances". To the best of my knowledge, the only data for grape, obtained however by a different methodological approach, are those of Flexas *et al.* (2002) indicating that in irrigated vines Cc was only about 50-60 % of Ci.

In our trials, at Ca = 400 ppm the ratio Cc/Ca was 0.46-0.48 which is fairly close to the values reported by Cornic and Fresneau (2002).

At Ca >500 ppm, Cc no longer increased, leading to an increasing divergence between values of Ci and Cc. Concurrently, g_{mes} declined significantly (-92 %).

When, in similar experiments, Ca values were raised to 2.000 ppm, Ci increased to 1.600 ppm while, again, Cc values increased at smaller rates reaching only about 10 % of the final Ci values. In parallel, g_{mes} declined about 6-fold reflecting a severe increase of the mesophyll resistance to the CO_2 transfer at high CO_2 supply. Meanwhile, responses of Cc and g_{mes} to alterations of ambient Ca and Ci have been confirmed for other *Vitis vinifera* cvs and *Helianthus annuus* (Düring, unpubl.).

These results indicate that increases of ambient CO_2 supply affect stomatal and mesophyll conductance to different degrees, thereby controlling the CO_2 transfer to the intercellular spaces and chloroplasts. It is hypothesised that both, stomatal and mesophyll conductance adapt the CO_2 supply to the actual CO_2 demand in chloroplasts.

This view is supported by experiments in which the CO_2 demand was raised by lowering ambient O_2 concentration from 21 % to 2 % (Table). As expected, photorespiration declined significantly (-69 %) leading to a small reduction of total photosynthetic electron flow. Similar results were obtained by CORNIC and BRIANTAIS (1991) reporting a decrease of electron transport at 1 % ambient O_2. Our data indicate that under non-photorespiratory conditions the increased demand for CO_2 was associated with higher stomatal and mesophyll conductances leading to increased CO_2 concentrations in chloroplasts.

T a b l e

Photosynthetic parameters under photorespiratory (21 % O_2) and non-photorespiratory (2 % O_2) conditions. Ambient CO_2 concentration: 400 ppm, leaf temperature: 25 °C, photosynthetic active radiation: 750 µmol m^{-2} s^{-1} (cv. Riesling)

Oxygen, %	g_{CO2}	Ci	g_{mes}	Cc	A	R_L	J_t
21	102	291	104	190	10.5	3.10	81
2	167	299	205	223	15.6	0.97	76

g_{CO2}, g_{mes} (mmol m^{-2} s^{-1}); Ci, Cc (ppm); A, R_L, J_t (µmol m^{-2} s^{-1}). See Material and Methods.

Conclusion

The presented data indicate that the relative low maximal CO_2 assimilation rates of grapevine leaves may, in fact, be explained by their relative high Ci-Cc difference, *i.e.* their high mesophyll resistance for CO_2. Moreover, the premise of LAWLOR and CORNIC (2002) - see Introduction - has been verified, since, in fact, increasing Ca altered mesophyll conductance. The data also raise questions, *e.g.* as to the site and mechanism of CO_2 sensing in leaves and the control of CO_2 transfer in the mesophyll. Determination of chloroplastic CO_2 concentration in grapevine genotypes will enable estimation of variety specific carboxylation efficiency and, for dehydrated vines, may contribute to elucidate causal relationships of factors limiting CO_2 assimilation.

References

BOTA, J.; FLEXAS, J.; KEYS, A. J.; LOVELAND, J.; PARRY, M. A.; MEDRANO, H.; 2002: CO_2/O_2 specificity factor of ribulose-1,5-bisphosphate carboxylase/oxygenase in grapevines (*Vitis vinifera* L.): First *in vitro* determination and comparison to *in vivo* estimations. Vitis **41**, 163-168.

CAEMMERER, S. VON; FARQUHAR, G. D.; 1981: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153**, 376-387.

CORNIC, G.; BRIANTAIS, J. M.; 1991: Partitioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C_3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrations and during drought stress. Planta **183**, 178-184.

CORNIC, G.; FRESNEAU, C.; 2002: Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during drought. Ann. Bot. **89**, 887-894.

DÜRING, H.; 1991: Determination of the photosynthetic capacity of grapevine leaves. Vitis **30**, 49-56.

DÜRING, H.; 1998: Photochemical and non-photochemical responses of glasshouse-grown grape to combined light and water stress. Vitis **37**, 1-4.

DÜRING, H.; DAVTYAN, A.; 2002: Developmental changes of primary processes of photosynthesis in sun- and shade-adapted berries of two grapevine cultivars. Vitis **41**, 63-67.

DÜRING, H.; LOVEYS, B. R.; 1996: Stomatal patchiness of field-grown Sultana leaves: Diurnal changes and light effects. Vitis **35**, 7-10.

EPRON, D.; GODARD, D.; CORNIC, G.; 1995: Limitation of net CO_2 assimilation rate by internal resistances to CO_2 transfer in the leaves of two tree species (*Fagus sylvatica* L. and *Castanea sativa* Mill.). Plant, Cell Environ. **18**, 43-51.

FARQUHAR, G. D.; SHARKEY, T. D.; 1982: Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. **33**, 317-345.

FLEXAS, J.; BOTA, J.; ESCALONA, J. M.; SAMPOL, B.; MEDRANO, H.; 2002: Effects of drought on photosynthesis in grapevines under field conditions: An evaluation of stomatal and mesophyll limitations. Funct. Plant Biol. **29**, 461-471.

HARLEY, P. C.; LORETO, F.; DI MARCO, G.; SHARKEY, T. D.; 1992: Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. Plant Physiol. **98**, 1429-1436.

KRALL, J. P.; EDWARDS, G. E.; 1992: Relationship between photosystem II activity and CO_2 fixation in leaves. Physiol. Plant. **86**, 180-187.

LARCHER, W.; 1975: Physiological Plant Ecology. Springer-Verlag Berlin.

LLOYD, J.; SYVERTSEN, J. P.; KRIEDEMANN, P. E.; FARQUHAR, G. D.; 1992: Low conductance for CO_2 diffusion from stomata to the sites of carboxylation in leaves of woody species. Plant, Cell Environ. **15**, 873-899.

LAWLOR, D. W.; 2002: Limitation to photosynthesis in water-stressed leaves: Stomata *vs.* metabolism and the role of ATP. Ann. Bot. **89**, 871-885.

LAWLOR, D. W.; CORNIC, G.; 2002: Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant, Cell Environ. **25**, 275-294.

NOBEL, P. S.; 1983: Biophysical Plant Physiology and Ecology. W. H. Freeman and Co., San Francisco.

ORTOIDZE, T.; DÜRING, H.; 2001: Light utilisation and thermal dissipation in light- and shade-adapted leaves of *Vitis* genotypes. Vitis **40**, 131-136.

RASCHKE, K.; 1975: Stomatal action. Ann. Rev. Plant Physiol. **26**, 309-340.

SCHREIBER, U.; 1997: Chlorophyllfluoreszenz und photosynthetische Energieumwandlung: Einfache einführende Experimente mit dem Teaching-PAM Chlorophyll Fluorometer. Heinz Walz GmbH.

VALENTINI, R.; EPRON, D.; DE ANGELIS, P.; MATTEUCCI, G.; DREYER, E.; 1995: *In situ* estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: Diurnal cycles under different levels of water supply. Plant, Cell Environ. **18**, 631-640.

WEYERS, J. D. B.; LAWSON, T.; 1997: Heterogeneity in stomatal characteristics. Adv. Bot. Res. **26**, 317-352.

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