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## Relationship between CO<sub>2</sub> assimilation and leaf anatomical characteristics of two grapevine cultivars

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**Abstract** – The possible implication of leaf anatomical characteristics on the photosynthetic rate was studied in two grapevine cultivars, Ribier (*Vitis vinifera* L.) and Isabella (*Vitis labrusca*), grown under field conditions. Ribier exhibited higher photosynthetic rates than Isabella, although there were no significant differences in the Rubisco activity and the stomatal conductance. The fraction of mesophyll volume represented by the intercellular spaces as well as the surface area of mesophyll cells exposed to intercellular air spaces were significantly lower in Isabella. Both gaseous CO<sub>2</sub> conductance through intercellular airspaces and liquid phase conductance were significantly higher in Ribier than in Isabella, contributing to a higher photosynthetic rate in this cultivar.

**leaf anatomy / mesophyll conductance / photosynthetic rate / *Vitis vinifera* / *Vitis labrusca***

**Résumé** – Relation entre l'assimilation de CO<sub>2</sub> et les caractéristiques anatomiques des feuilles de deux espèces de vigne. L'influence possible des caractéristiques anatomiques des feuilles sur le taux de photosynthèse a été étudiée pour deux espèces de vigne : *Vitis Labrusca* (Isabella) and *Vitis vinifera* L. (Ribier), cultivées en culture au champ. Ribier a présenté un taux photosynthétique plus élevé que Isabella, bien qu'il n'y ait pas de différences significatives de l'activité de la Rubisco et de la conductance stomatique. La fraction du volume de mésophylle représentée par l'espace intercellulaire, ainsi que la surface des cellules du mésophylle exposée à l'air dans l'espace intercellulaire, étaient significativement plus faibles chez Isabella. Les conductances gazeuses pour le CO<sub>2</sub> à travers l'espace intercellulaire et en phase liquide étaient significativement plus fortes pour Ribier que pour Isabella, contribuant à des taux photosynthétiques plus importants pour cette espèce.

**anatomie des feuilles / conductance interne / taux photosynthétique / *Vitis vinifera* / *Vitis labrusca***

### 1. INTRODUCTION

It is well known that, under non-limiting environmental conditions for gas exchange by leaves, rates of CO<sub>2</sub> assimilation may be regulated by the intrinsic photosynthetic capacity of the mesophyll and by CO<sub>2</sub> transfer conductances from ambient air to carboxylation sites within chloroplasts [11, 12]. These conductances include boundary layer, stomatal ( $g_s$ ) and internal conductance ( $g_i$ ) within the mesophyll. The first two conductances could be accurately estimated by measurements of transpiration rates. On the other hand, changes in leaf anatomical characteristics are known to alter the internal CO<sub>2</sub> conductance from the substomatal cavities to sites of carboxylation and thus the photosynthetic rate [7]. A strong correlation between CO<sub>2</sub> internal conductance and photosynthetic

rate has been found both within and between species [11]. However, the relative importance of internal conductance in determining the photosynthetic rate in grapevine cultivars remains unknown.

Differences in photosynthetic capacity are of great importance as they are closely related to plant productivity. Photosynthetic rate is known to vary considerably in different grapevine cultivars [3, 4, 5, 20]. The reasons for the differential photosynthetic behavior in these cultivars remain more or less obscure.

The aim of this study was to determine the leaf anatomical and physiological differences between two grapevine cultivars and evaluate whether photosynthetic rate could be related to their leaf anatomical characteristics.

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## 2. MATERIALS AND METHODS

Fifteen-year-old field-grown grapevines of *Vitis vinifera* L. cv. Ribier and *Vitis labrusca* cv. Isabella were used. The soil was a clay loam, 17% clay, 36% silt, 47% sand, CEC = 14.5 cmol·kg<sup>-1</sup>, 11% CaCO<sub>3</sub> and a field capacity of 18.5% w/w. The plants were grafted onto 110R (*V. berlandieri* × *V. rupestris*) rootstock and were cordon-trained in a bilateral cordon. Vine spacing was 1.8 m in rows and 2.5 m apart. All plants were uniformly pruned in the winter with a load of 12 buds per plant. Approximately one month after bud break all the plants were thinned to one cluster per shoot. Electronic tensiometers, placed at 25 cm depth, were used for monitoring soil water potential. In order to maintain high water availability in the soil, drip irrigation was applied when soil water potential reached -0.03 MPa (average value from 10 tensiometers), approximately two times every week. Starting in May and continuing throughout the season, predawn and midday leaf water potential ( $\Psi$ ) as well as osmotic potential ( $\Pi$ ) were determined every ten days. These measurements were performed on three mature leaves per treatment, at each sampling time during the day, using the psychrometric technique [15]. Two pairs of leaf discs were obtained from each one of the three leaves. The first pair of discs was used for leaf water potential determination while osmotic potential was measured on the second pair. Turgor potential (P) was estimated using the equation [9]:

$$P = \Psi - \Pi \quad (1)$$

Diurnal measurements of gas exchange parameters were made four times during the growing season, at ten-day intervals, starting 15 days after berry set. These measurements were performed every two hours, from 06:00 to 18:00 h, on ten fully expanded mature exterior leaves per treatment, at saturation light intensity, using a portable gas exchange system (Li-6400, Li-Cor Inc, Nebraska USA). The leaves that were used for these measurements were 40–50 days old. In order to estimate exactly the leaf age, leaf unfolding was systematically recorded throughout the growing season. The values of gas exchange parameters measured early in the morning – approximately at 10:00 pm – were considered as maximum diurnal values. Concomitant measurements of the maximum rate of Rubisco activity ( $V_c$ ) were also performed on the leaves that were used for the maximum  $P_n$  determination. This was calculated using the equation [13, 14]:

$$V_c = (P_n + R_d)(C_i + K_m)/(C_i - \Gamma) \quad (2)$$

where  $P_n$  is the maximum diurnal values of photosynthetic rate,  $K_m$  is the effective Michaelis-Menten constant for CO<sub>2</sub> (46 Pa according to Farquhar et al. [6]),  $\Gamma$  is the compensation point of CO<sub>2</sub> in the absence of respiration [17], and  $C_i$  the intercellular CO<sub>2</sub> concentration. The rate of respiration ( $R_d$ ) was measured according to the gas exchange measurements protocol with the only exception that the sample cuvette was enclosed in aluminum foil to exclude light [16].

Chlorophyll content was estimated by absorbance at 645 and 663 nm of an 80% acetone extract [1] of ten leaf discs

obtained from each leaf used for gas exchange measurements each sampling time.

For anatomical measurements, ten sections obtained from each of ten mature fully expanded exterior leaves from each treatment were examined under a scanning electron microscope. Five of the best transverse sections without folds were photographed to be used for anatomical measurements. Photographs were taken using a transmission electron microscope attachment and printed in four to eight electron micrographs. These micrographs were pieced together to yield a composite photomicrograph of sufficient enlargement to allow measurement of the surface areas of individual chloroplasts using a BioQuant IV electronic digitizer. Total leaf thickness (L) and mesophyll thickness (l), as well as the width of sections (w) were also measured. The cross-sectional length of mesophyll tissue exposed to intercellular air spaces was measured ( $L_m$ ), and the surface of mesophyll cells exposed to the intercellular air spaces per unit leaf area ( $S_m$ ) was calculated as [18]:

$$S_m = 1.34 (L_m/w) \quad (3)$$

The factor 1.34 accounts for cell surfaces that were not uniformly perpendicular to the plane of the section based on the average width to height ratio for palisade and spongy cells [7, 19].

The fraction of mesophyll tissue occupied by the intercellular air spaces ( $f_{ias}$ ) was determined as:

$$f_{ias} = 1 - A_m/lw \quad (4)$$

where  $A_m$  is the total cross-sectional area of mesophyll cells per unit leaf area and l is the mesophyll thickness between the upper and lower leaf epidermises.

An estimate of gaseous conductance through the intercellular air spaces ( $g_{ias}$ ) was obtained using the equation [18]:

$$G_{ias} = (f_{ias})^{1.55} / lk \quad (5)$$

where the 1.55 power accounts for a modeled tortuosity in the diffusion path through small pores [2], and k is a fitted constant, which for simplicity was considered as equal to one in both cultivars.

Leaf tissue density ( $T_d$ ) was determined as [18]:

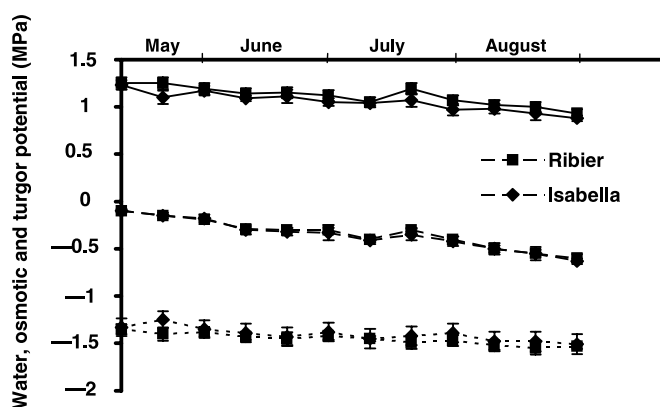
$$T_d = (D/a) / L(1 - f_{ias}) \quad (6)$$

where L is the total leaf thickness and D/a represents the leaf dry weight (D) per unit leaf area (a).

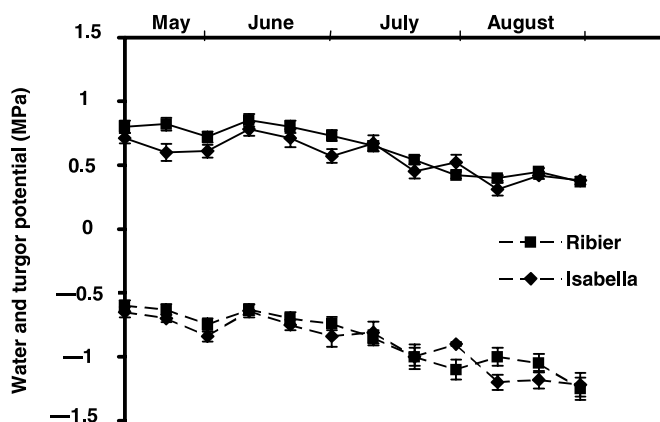
A completely randomized experimental design was used. Statistical analysis was done using the SPSS statistical computer package (SPSS for Windows, standard version, release 6.1). ANOVA and mean separation by LSD test were used to compare the treatments.

## 3. RESULTS AND DISCUSSION

Seasonal values of predawn and midday leaf water potential ( $\Psi$ ) decreased continuously from May to August with no significant differences between the two cultivars (Figs. 1 and 2).

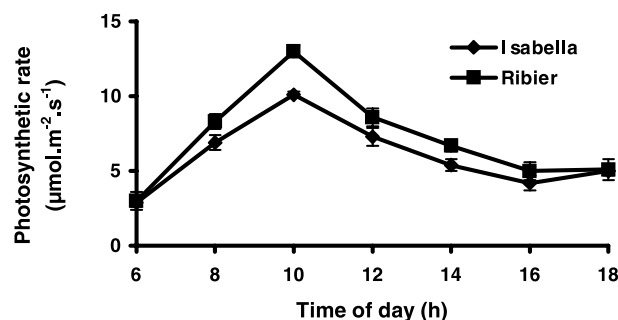


**Figure 1.** Seasonal changes in predawn water potential (-----), osmotic (.....) and turgor potential (solid line) in the two cultivars. Vertical lines indicate the standard error of six replicates for each of the two cultivars.

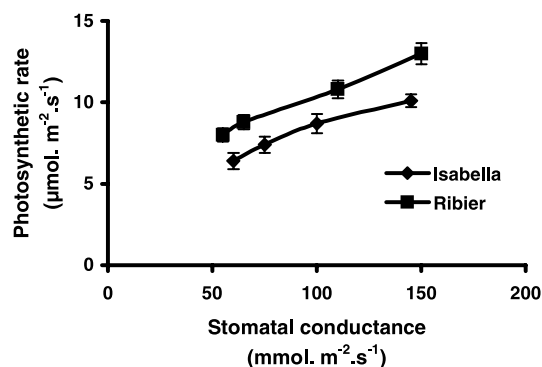


**Figure 2.** Seasonal changes in midday water potential (-----) and turgor potential (solid line) in the two cultivars. Vertical lines indicate the standard error of six replicates for each of the two cultivars.

Also, the seasonal changes in predawn and midday osmotic potential ( $\Pi$ ) followed the same pattern, resulting in turgor maintenance in both cultivars (Figs. 1 and 2). Diurnal values of gas exchange parameters also did not significantly differ during the period in both cultivars (data not shown) and thus the results presented here (Fig. 3) refer to the means of all the measurements. The maximum values of photosynthetic rate – obtained early in the morning – were significantly higher in Ribier compared with Isabella (Fig. 3). Lower values of photosynthetic rate in Isabella can be attributed to: (i) stomatal limitations; (ii) biochemical constraints on  $\text{CO}_2$  assimilation, and/or (iii) differences in internal conductance for  $\text{CO}_2$  diffusion from the stomatal cavity to chloroplasts [7, 10]. The relationship between maximum diurnal  $P_n$  and stomatal conductance ( $C_s$ ) revealed that Ribier exhibited higher values of  $P_n$  for the same values of  $C_s$  (Fig. 4). Since no differences in stomatal conductance between the two cultivars occurred, we can assume that the lower values in the photosynthetic rate in Isabella can



**Figure 3.** Diurnal changes in photosynthetic rate ( $P_n$ ) in the two cultivars. Vertical lines indicate the standard error of 40 replicates for each of the two cultivars.



**Figure 4.** Relationship between leaf stomatal conductance to water vapor and photosynthetic rate in the two cultivars. Vertical lines indicate the standard error of 40 replicates for each of the two cultivars.

be attributed to either biochemical or anatomical limitations. As far as the possible biochemical constraints on  $\text{CO}_2$  assimilation are concerned, current biochemical models of photosynthesis consider that  $\text{CO}_2$  assimilation is limited by the activity of ribulose 1,5-biphosphate carboxylase/oxygenase (Rubisco) and/or chlorophyll content [8, 11]. However, the estimated values of maximum Rubisco activity and the chlorophyll content did not significantly differ in the two cultivars (Tab. I). Thus, lower values of photosynthetic rate in Isabella do not seem to be related to Rubisco activity. This inability of either stomatal or biochemical limitations to account for the low  $\text{CO}_2$  assimilation rate leads us to suggest that probably a low internal

**Table I.** Changes in maximum photosynthetic rate, Rubisco activity and chlorophyll content in the two grapevine cultivars. Data are means  $\pm$  S.E. of 40 replicates.

	Photosynthetic rate ( $P_n$ ) ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Rubisco activity ( $V_c$ ) ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Chlorophyll content ( $\text{mg}\cdot\text{dm}^{-2}$ )
Ribier	$13.1 \pm 0.7^*$	$36.3 \pm 5.2$	$3.51 \pm 0.15$
Isabella	$10.1 \pm 0.5$	$33.9 \pm 4.7$	$3.47 \pm 0.09$

\*  $P < 0.05$ .

**Table II.** Leaf anatomical properties of the two grapevine cultivars ( $f_{ias}$  = the fraction of mesophyll tissue occupied by the intercellular air spaces,  $g_{ias}$  = gaseous conductance through intercellular air spaces,  $T_d$  = leaf tissue density and  $S_m$  = surface area of mesophyll cells exposed to the intercellular air spaces per unit leaf area). Data are means  $\pm$  S.E. of 10 replicates.

	$f_{ias}$	$g_{ias}$ ( $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) $\times 10^{-4}$	$T_d$ ( $\text{g} \cdot \text{m}^{-3}$ ) $\times 10^5$	$S_m$ ( $\text{m}^2 \cdot \text{m}^{-2}$ )
Ribier	0.4 $\pm$ 0.06 **	15.39 $\pm$ 2.5 **	7.52 $\pm$ 0.8	15.2 $\pm$ 1.1 **
Isabella	0.28 $\pm$ 0.04	8.96 $\pm$ 1.2	6.27 $\pm$ 0.6	12.5 $\pm$ 0.8

$P < 0.05$ , \*\*  $P < 0.01$ .

conductance ( $g_j$ ) for  $\text{CO}_2$  diffusion from the stomatal cavity to chloroplasts could be responsible for the lower values of  $P_n$  in leaves of Isabella. Internal conductance is considered as the sum of two components: an intercellular gas phase conductance from the stomatal cavity to outer surfaces of mesophyll cell walls ( $g_{ias}$ ) and a liquid-phase conductance across cell walls to sites of carboxylation within chloroplasts ( $g_{cw}$ ) [10, 14].

The values of both components of the total internal conductance are known to be closely related to leaf anatomical features [10, 18]. The measurements of leaf anatomical characteristics showed significant differences between the two cultivars (Tab. II). The fraction of mesophyll volume represented by intercellular air spaces ( $f_{ias}$ ) was significantly greater in Ribier, resulting in almost double values of the calculated gaseous conductance through the intercellular air spaces ( $g_{ias}$ ) (Tab. II). High  $g_{ias}$  means more rapid diffusion of  $\text{CO}_2$  from the substomatal cavity to the cell wall surfaces, a fact that may explain the higher photosynthetic rate in Ribier, despite the similar values of stomatal conductance. On the other hand, the liquid face conductance ( $g_{cw}$ ) includes the  $\text{CO}_2$  flow across the cell walls and across cytoplasmic and chloroplastic membranes, and thus it is much more difficult to be estimated [14]. However, reasonable estimates of  $g_{cw}$  could be obtained from the average tissue density as well as the surface of the mesophyll cells exposed to intercellular air spaces ( $S_m$ ) [18]. Our results show no significant differences in leaf tissue density despite the differences in  $f_{ias}$  (Tab. II), a fact that might be attributed to: (i) the difference in the number of mesophyll cells per unit of leaf area, and/or (ii) to differences in cell wall thickness between the two cultivars. Since no significant difference in the number of mesophyll cells per unit of leaf area between the two cultivars occurred (data not shown) we can suppose that cell wall thickness would be lower in Ribier compared with Isabella. A less thick cell wall shortens the path from the outer surface of the cell walls to the sites of carboxylation over which the  $\text{CO}_2$  must diffuse, resulting in higher  $g_{cw}$ . Furthermore, the surface of mesophyll cells exposed to the intercellular air spaces per unit leaf area ( $S_m$ ) was also significantly greater in Ribier (Tab. II). These data provide evidence that  $g_{cw}$  might be greater in Ribier, contributing to the higher photosynthetic rates of this cultivar.

#### 4. CONCLUSION

Our data confirm that differences in photosynthetic rate in the two grapevine cultivars could be attributed to their differ-

ences in leaf anatomical characteristics, which affected the total internal  $\text{CO}_2$  conductance.

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