Low air humidity causes non-uniform stomatal closure in heterobaric leaves of Vitis species

by

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Niedrige Luftfeuchte verursacht uneinheitlichen Stomataschluß bei heterobarischen Blättern von Vitis-Arten


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Introduction

Only a few years ago, leaf gas exchange was evaluated assuming that photosyn¬thetic activity was uniform in all parts of a leaf. However, results of ISHIHARA et al. (1971), LAISK et al. (1980) and TERASHIMA et al. (1988) revealed strong evidence that there exists a wide variation in aperture of stomata even for the small area of leaves of Oryza sativa or Hordeum vulgare. Direct microscopic analysis of individual stomata indicates that even neighbouring stomata behave differently when subjected to envi¬ronmental stress (OMASA et al. 1985; KAPPEN et al. 1987). Also, groups of fully closed sto¬mata were found in the vicinity of groups of fully open stomata (LAISK 1983). Such a non-uniform stomatal behaviour is obviously related to the anatomy of heterobaric leaves. According to NEGER (1918) the mesophyll of heterobaric leaves is separated into intercellular chambers hermetically sealed other than their own stomatal openings, while homobaric leaves have no barriers to gas exchange within the mesophyll.

Since the conventionally used calculation of the internal CO₂ partial pressure (p_i) from gas exchange measurements implies the assumption of uniformly open stomata on the leaf blade, the occurrence of non-uniform stomatal behaviour must lead to erro¬neous results (VON CAEMMERER and FARQUHAR 1981; TERASHIMA et al. 1988; VAN KRALINGEN 1990). DOWNTON et al. (1988 a and b, 1990) were the first to demonstrate non-uniform stomatal behaviour in leaves of grapevines (Cabernet Sauvignon, Sultana) after infiltration of abscisic acid (ABA) and as a consequence of water deficiency or salt stress.

In the present study evidence is presented for the predominance of the heterobaric leaf type in Vitis spp. It will be demonstrated that a decrease of the photosynthetic
activity under conditions of low air humidity is caused by stomatal limitation of CO₂ uptake and not - as was previously assumed - by mesophyll factors.

Material and methods

Fully expanded field-grown leaves of *V. berlandieri*, *V. rupestris*, *V. riparia*, *V. amurensis*, *V. slavini*, *V. doaniana*, *V. andersonii* and *V. shuttleworthii* were taken from the grapevine collection of the Institute of Grapevine Breeding Geilweilerhof at Siebeldingen. 3-year-old ungrafted Müller-Thurgau vines grown in pots and cultivated in the substrate 'Einheitserde P' in the glasshouse were transferred to the laboratory 1 week before the experiments were started. They were kept at 24—25 °C (day, 14 h) and 18—20 °C (night), respectively. Light was provided by 2 Osram, HQI-T 400 W/DH lamps with ca. 800 µmol quanta · m⁻² · s⁻¹ at the apical leaves and 300—350 µmol quanta · m⁻² · s⁻¹ at the basal leaves. Relative air humidity was about 50 %. Gas exchange was measured using a 'Miniküvetten-System' (Fa. Walz, Effeltrich, BRD) at 35 Pa CO₂, light saturation (750 µmol quanta · m⁻² · s⁻¹) and 26 °C leaf temperature. Leaf conductance of fully expanded leaves was determined at various leaf to air water vapour pressure differences (Δw) (for details: DURING 1988, 1991). When, after altering Δw, photosynthesis and transpiration rates had reached a steady state, the circular leaf area (11.94 cm²) inserted into the cuvette was punched out and immediately weighed (W₁).

Subsequently this leaf disc was subjected to a water infiltration procedure (MOLISCH 1912; MICHAEL 1969; BEYSCHLAG and PFANZ 1990). The leaf disc was placed into a plastic syringe (50 ml) which had been filled before with about 30 ml of distilled water. Remaining air bubbles were removed through the outlet of the syringe. The outlet was then closed and a vacuum was created in the syringe by pulling the piston outward. Simultaneously the syringe was shaken to remove air from internal leaf spaces. After having released this air, the syringe was connected to an electronic pressure meter (DPM 35-G, Penwalt, BRD); the pressure inside the syringe was increased to 0.30 MPa by pushing the piston into the syringe. By this procedure water penetrated the leaf via open stomatal pores. The leaf disc was then blotted dry by filter paper and weighed again (W₂). From W₁ and W₂ the amount of water taken up by the leaf disc can be estimated. The backlit lower leaf surface was photographed and the photograph copied to an overhead foil. Using a leaf area meter (LI-3100, Li-COR, USA), the dark (non-infiltrated) areas of the disc were determined and related to its total area.

The reaction of gas exchange to changes of Δw was determined by a stepwise lowering of the dew point in the cuvette to values < 21 °C.

All measurements were performed from 8 to 11 a.m. The gas exchange data were calculated according to VON CAEMMERER and FARQUHAR (1981); the intercellular CO₂ partial pressure derived from gas exchange data (pᵢ-IRGA) was corrected according to a proposal by DOWNTON et al. (1988 b). The corrected pᵢ can be obtained approximately by

\[ \text{pᵢ-CORR} = R \times (pᵢ-\text{IRGA}) + (1-R)Γ \]

where R is the ratio of photosynthetic rates (A) at the same pᵢ (R = A-stress/A-control), and Γ is the CO₂ compensation point. In our experiment Γ was 4.7 Pa. The carboxylation efficiency (CE) was calculated as follows:

\[ \text{CE} = A/(pᵢ-Γ). \]
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Results and discussion

In a previous paper, it has already been shown that decreasing air humidity can induce stomatal closure (DORING 1987). Using this dependency in a series of experiments with Müller-Thurgau vines, circular leaf areas were subjected either to \( \Delta_w = 14.2 \text{ Pa} \cdot \text{kPa}^{-1} \) (corresponding to 58% RH at 26°C) or to \( \Delta_w = 26.0 \text{ Pa} \cdot \text{kPa}^{-1} \) (corresponding to 23% RH at 26°C). After water infiltration the leaves were photographed using backlight; light areas indicate infiltrated, dark areas non-infiltrated parts. As is demonstrated by the light circle (centre of Fig. 1 A), at high air humidity a large number of open stomata enable water infiltration. The dark ring around the light circle is caused by the India rubber ring which is pressed on the leaf blade to seal the cuvette air from ambient air; stomata situated on this ring are almost entirely closed. At lower air humidity only groups of stomata are open, light areas with open stomata can be clearly distinguished from dark areas, where stomata are closed (Fig. 1 B). Fig. 1 C shows a wilted leaf; most of the stomata are closed (\( g_{H_2O} = 36 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) and subsequently dark areas predominate.

These results clearly demonstrate that the stomatal behaviour of Müller-Thurgau leaves at low air humidity is non-uniform confirming the heterobaric leaf type of \( V. \) vinifera. Additional experiments on the behaviour of stomata under low air humidity conditions were performed using leaves of \( V. \) berlandieri, \( V. \) rupestris, \( V. \) riparia, \( V. \) amurensis, \( V. \) slavini, \( V. \) doaniana, \( V. \) andersonii and \( V. \) shuttleworthii. Leaves of all these genotypes exerted 'patchiness' similar to that shown in Fig. 1. Thus, it appears that the heterobaric leaf type is a widespread character within the genus \( Vitis. \)

A quantitative evaluation of the photographs of leaf discs after water infiltration indicates that the portion of the infiltrated (light) area increases with leaf conductance determined by porometry (data not shown). In addition, the amount of infiltrated water was positively correlated with leaf conductance (Fig. 2), indicating that the reduction of water infiltration at low air humidity was in fact caused by stomatal closure. To our surprise even at leaf conductance values close to zero about \( 0.5-1 \text{ mg H}_2\text{O} \cdot \text{cm}^{-2} \) was infiltrated. In experiments with entire leaf blades (the cut ends of petioles were closed by wax) no water was infiltrated at leaf conductance close to zero. Thus it is assumed that some water may have entered the leaf blade via the peripheral, injured tissue.

If we assume that in the light areas of the infiltrated leaf discs stomata are more or less homogeneously open and closed in the non-infiltrable dark areas, it results that the conventional calculation of the intercellular \( CO_2 \) partial pressure (\( p_\text{i}-\text{IRGA} \)) is correct in areas where stomata are open, while in areas where stomata are closed \( p_\text{i} \)-values are close to the \( CO_2 \) compensation point. Non-uniform stomatal closure therefore causes an overestimation of \( p_\text{i} \)-values (TERASHIMA et al. 1988; DOWNTON et al. 1988 a and b). This is demonstrated using gas exchange data obtained under different air humidity conditions (Table).

A decrease of air humidity lowered rates of leaf conductance (\( -22 \% \)) and photosynthesis (\( -27 \% \)). From a series of measurements gas exchange data with identical \( p_\text{i}-\text{IRGA} \) values were selected. Calculation of the carboxylation efficiency (\( A/p_\text{i} \) or CEC-IRGA) indicates reduced values at low air humidity. In the past it was deduced that under stress conditions, besides stomatal limitations of photosynthesis, non-stomatal factors would reduce photosynthetic activity (Review: SCHULZE 1986). A more realistic estimation of \( p_\text{i} \) under stress conditions which takes into consideration the non-uniform stomatal behaviour was proposed by DOWNTON et al. (1988 b). At low air humidity this corrected \( p_\text{i} \)-value (\( p_\text{i}-\text{CORR} \)) decreased compared to \( p_\text{i}-\text{IRGA} \) and CE-CORR at low air humidity differs only slightly from CE-IRGA at high air humidity (Table). Thus, lowering air humidity obviously did not alter mesophyll factors; rather it is
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evident that reductions of photosynthesis are caused by stomatal limitation of CO₂ uptake.

Fig. 2: The amount of infiltrated water related to leaf conductance (gH₂O) of leaf discs. r = 0.88.

Die Menge an infiltriertem Wasser bezogen auf die Blattleitfähigkeit (gH₂O) von Blattscheiben. r = 0.88.

Conclusion

The results on stomatal behaviour obtained so far in experiments with grapevine leaves have clearly revealed non-uniform stomatal closure due to certain stress factors or infiltrated ABA. As a consequence, estimations of p₅ from gas analysis data under stress conditions will lead to erroneous results and values of carboxylation efficiency.
are underestimated. This rather new aspect calls in question former results concerning CE but also provokes new questions.

E.g., is the occurrence of patches really a temporary phenomenon which disappears if the ambient conditions remain constant for a sufficient enough time (BEYSCHLAG and PFANZ 1990) or, are gas tight compartments in the mesophyll of leaves of ecological significance for the inhibition of the diffusion of fumes into the entire leaf blade (NEGER 1918)? There are certainly more aspects which would deserve further investigation.

Rate of photosynthesis (A), leaf conductance for CO₂ (gCO₂), intercellular CO₂ partial pressure (pᵢ) and the carboxylation efficiency (CE) of Müller-Thurgau leaves as affected by low and high leaf to air water vapour pressure difference (Δw). Note that a low Δw value corresponds to a high relative humidity (RH). IRGA = derived from gas exchange data, CORR = corrected according to DOWNTON et al. (1988 b)

| Δw, Pa · kPa⁻¹ | 14.2 | 26.0 |
| RH, %          | 36.0 | 23.0 |

Summary

Water infiltration experiments indicate that cv. Müller-Thurgau and several Vitis spp. belong to the heterobaric leaf type which is characterised by airtight intercellular chambers in the mesophyll. Stomatal closure of heterobaric leaves was non-uniform if leaves were exposed to low air humidity.

Leaf conductance was correlated with the infiltrated leaf area and the amount of infiltrated water. Taking into account non-uniform stomatal closure of vine leaves in the calculation of intercellular CO₂ partial pressure (pᵢ) (DOWNTON et al. 1988 b), pᵢ was reduced at low air humidity. The almost identical carboxylation efficiency (A/pᵢ) under moist and dry air conditions indicates that the decline of the photosynthetic rate caused by dry air is entirely due to stomatal limitation of CO₂ uptake.

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Literature


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