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Rapid stomatal and photosynthetic responses of Vitis berlandieri leaves after petiole excision in water

by

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S u m m a r y : Excision of petioles of *Vitis berlandieri* leaves in water caused a transient decline of stomatal conductance within 30-50 s at high and low leaf water potentials. The time of the subsequent recovery of stomatal conductance to the starting point increased with decreasing leaf water potentials ($r = -0.969$). It is concluded that the rapid increase of pressure in xylem vessels after excision in water is transmitted directly to the epidermis and the stomata bypassing the mesophyll cells.

The transient decrease of photosynthesis observed briefly after petiole excision in water is caused by pressure changes in xylem and by transient stomatal closure and not — as has been shown for other species — by inhibitory solutes originating from the wounded cells of the petiole. This is demonstrated by (1) the instantaneous reversibility of the transient reactions of stomata and photosynthesis by withholding water, (2) the hydraulic signals moving in apical *and* basal direction in vines, (3) the photosynthesis to stomatal conductance ratio being constant during the experiment, (4) the determination of the dark respiration which remained constant after excision of petioles.

K e y w o r d s : *Vitis*, xylem, water potential, stomatal conductance, photosynthesis

Introduction

During transpiration of grapevine leaves the water columns in the xylem system are under tension and normally suffer diurnal cycles even under favourable ambient conditions (Düring and Loveys, 1982). It is assumed that abrupt changes in water supply to leaves as brought about by leaf excision in the *air* will cause pressure changes in xylem and shrinkage of epidermal cells permitting a transient expansion of the guard cells, i.e. stomatal opening. This stomatal reaction has been described by Darwin (1897) and Iwanoff (1928) and was named the 'Iwanoff effect'. When the petiole of a transpiring leaf is excised in *water* the rapid access of water to the leaf tissue presumably causes swelling of the epidermal cells thereby compressing and closing the guard cells (Raschke 1970). Studies on these transient reactions, to our knowledge, have not yet been performed in grapevines; they may contribute to elucidate the poorly understood relation between bulk leaf water potential and stomatal aperture and the water transport in leaves as well.

Stomatal closure induced by petiole excision in water was associated with transient inhibition of photosynthesis of leaves of *Lycopersicon esculentum* while in leaves of *Arbutus unedo* and *Helianthus annuus* stomatal closure was insufficient to account for transient inhibition of photosynthesis (Heber *et al.* 1986). Obviously in these two species a yet unknown inhibitory solute is transported from the excised end of the petiole to the mesophyll where it induces a transient reduction of photosynthesis and an increase of dark respiration (Heber *et al.* 1986, Gsell *et al.* 1989).

The aim of the presented experiments was to study the effect of leaf water potential on stomatal reactions after petiole excision in water and to find out whether in *Vitis*, like in *Arbutus* and *Helianthus*, an inhibitory solute is involved in the transient inhibition of photosynthesis.

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Material and methods

Plant material and petiole excision in water. 3-year-old potted *Vitis berlandieri* plants grown outdoors were decapitated above the 13th leaf a couple of days before the experiments were started. Plastic tubes (inner diameter: 2 cm, length: 5 cm) were cut at one side in a longitudinal direction; both ends of each tube were sealed by foam rubber discs (diameter: 2 cm, thickness: 1 cm) (Fig. 1). The discs which were glued into the inner part of the plastic tubes were cut in by a razor-blade (depth: 1 cm) at the longitudinal cut of the tube so that part of the leaf petiole could be inserted in the tube. The ends of the tube were gently pressed to the petiole by twisting a metal wire around the tube at each side in order to avoid water leakage after filling the tube with tap water. Excision of the petioles was performed in water using stainless steel scissors.

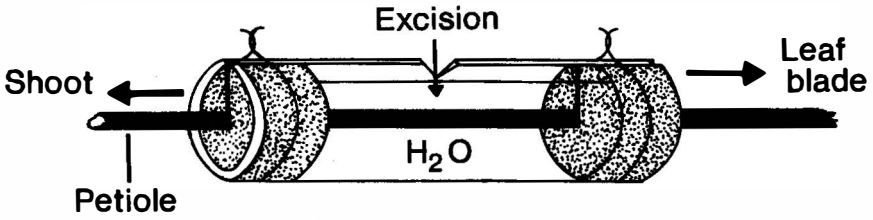

Fig. 1: Leaf petiole excision in water.

Gas exchange and water potential measurement. Part of the leaf blade (0.001 m^2) at the distal end was inserted into a cuvette chamber of the Minicuvette-system (H. Walz, D-8521 Effeltrich, Germany) and sealed by a lid containing two layers of glass. Inside temperature was held at 26 ± 1 °C, the leaf to air water vapor pressure difference of the entering air was kept constant (dew point: 13 °C).

Illumination was provided for the enclosed part of the leaf by a 12 V 75 W projector lamp (General Electric) using KG 1 and NG 11 filters (H. Walz). Light intensity at the leaf surface was about 1200 µmol quanta · m^{-2} · s^{-1} to measure photosynthesis at light saturation (DÜRING 1988).

CO_2 concentration of the entering air was kept constant at 350 ppm by a gas mixing device (H. Walz). Exchange of CO_2 and H_2O vapor was recorded by an IR differential gas analyser (Binos, Leybold Heraeus, Hanau, Germany). To eliminate errors in CO_2 measurement due to cross sensitivity to water vapor the measuring and reference air streams after transpiration measurement passed through a water vapor trap (dew point: 2 °C). During the experiments gas exchange was recorded at intervalls of 10 s; these results were stored by a data logger (DES-12, H. Walz). The calculation of gas exchange parameters is based on the propositions made by VON CAEMMERER and FARQUHAR (1981). Water potential of leaves was determined using the pressure chamber technique (SCHOLANDER *et al.* 1965).

Results and discussion

1. Effects of leaf water potential

In Fig. 2 typical examples of stomatal reactions to petiole excision in water are presented. At high and low leaf water potentials stomatal conductance decreased rapidly, about 30-50 s after petiole excision. It is interesting to note that at high leaf water potential the starting-point of stomatal conductance was reached again ca. 30 min after excision while at low leaf water potential it was reached only after about 75 min. Similar experiments performed at different

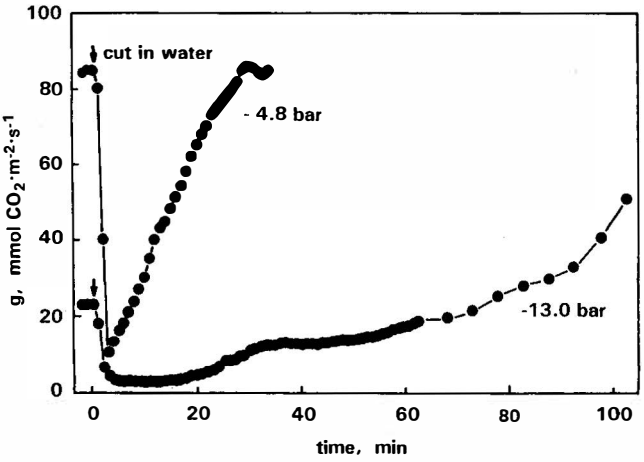

Fig. 2: Effects of petiole excision in water on stomatal conductance (g) of leaf blades at high and low leaf water potentials.

leaf water potentials revealed a close relationship (r = -0.969) between leaf water potential and the time from petiole excision to recovery of stomatal conductance (Fig. 3). Because of the high resistance to water fluxes in the mesophyll the rapid stomatal response to petiole excision was unexpected, especially at low leaf water potentials (TYREE and CHEUNG 1977; BOYER 1985). However, there are some indications of close (vascular) connections between leaf xylem and epidermis in which stomata are imbedded (SHERIFF and MEIDNER 1974); thus in our experiments water influx may possibly have bypassed at least part of the mesophyll cells (BOYER 1985). This view is strengthened by results of NONAMI and SCHULZE (1989) who measured distinct differences in water potential and turgor between mesophyll and epidermis cells in *Tradescantia* leaves with the cell pressure probe. Delayed recovery of stomatal conductance at low leaf water

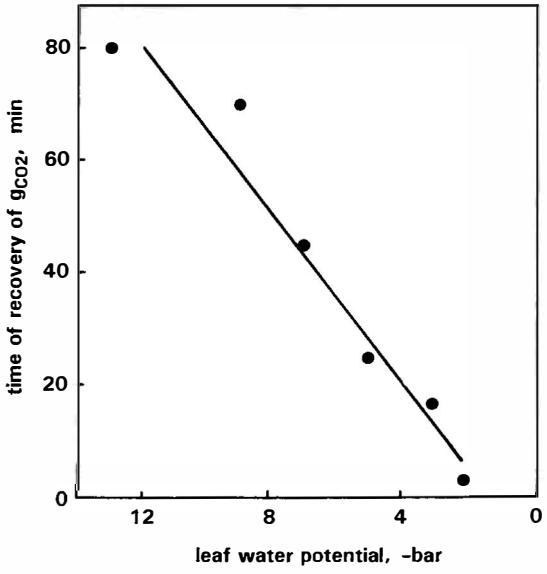

Fig. 3: The relationship between the time of recovery of stomatal conductance (gco_2) after excision of petioles in water and the leaf water potential (r = -0.969).

potentials has been observed in grapevines and some other species also. The delay possibly indicates a *qualitative* difference between recovery at high and low water potential, although the underlying mechanism are unclear (for discussion: MANSFIELD and DAVIES 1981).

2. Control of photosynthesis after excision

In Fig. 4 the dotted line denotes reactions of stomatal conductance (g) and photosynthesis (A) of leaf blades just before and after excision of their petioles in water. In most experiments stomatal conductance and photosynthesis declined synchronously; in 3 out of 15 experiments stomata reacted shortly before the rates of photosynthesis decreased. Ca. 7 min after excision, stomatal conductance and photosynthesis started to increase again to reach the starting-point after about 10 min. Although in this typical example the courses of stomatal conductance and of photosynthesis appear to be running in parallel it may be argued that water soluble solutes transported by the transpiration stream from the site of excision to the leaf blade may have transiently inhibited photosynthesis (GSELL *et al.* 1989). It was anticipated that in the case of a *hydraulic* chain of events a sudden decrease of xylem and epidermal pressure should lead to an almost instantaneous increase of photosynthesis due to stomatal opening.

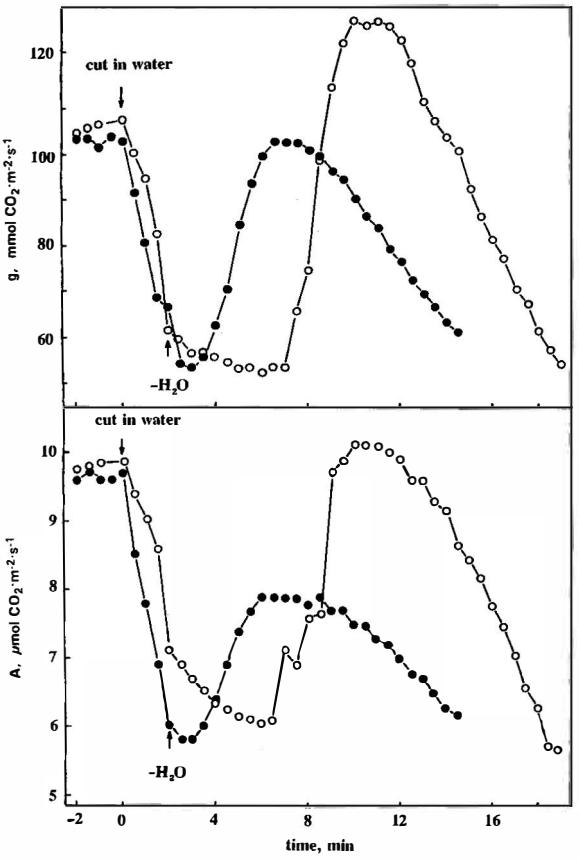

Fig. 4: Photosynthesis (A) and stomatal conductance (g) of leaves after excision of their petioles in water. *Dotted line*: excision enables a continous access of water to the leaf blade throughout the experiment; *solid line*: the access of water to the leaf blade is interrupted. Note that the removal of the water reservoir ca. 100 s after excision ($-H_2O$) induces a rapid increase of g ('IWANOFF effect').

Therefore we excised petioles in water, but this time the water filled tube was disconnected from the excised leaf ca. 120 s after excision (Fig. 4: solid line, arrow -H_2O). Only about 60 s later this interruption of water supply led to a synchronous increase of photosynthesis and stomatal conductance ('IWANOFF effect'); thus photosynthesis does not appear to be inhibited by metabolites originating from wounded cells.

More evidence for the hydraulic nature of the transient inhibition of photosynthesis in grapevines was obtained by experiments in which the signal derived from excision in water could hardly have been transported by the transpiration stream: After excision of the petiole of an apical leaf (Nr. 13) we recorded photosynthetic and stomatal reactions of leaf Nr. 7, i.e. 1.4 m below the apical leaf. Obviously changes in xylem pressure are rapidly transmitted in apical *and* basal direction to other organs. In all experiments with *Vitis* the extent of transient inhibition of photosynthesis corresponded well with the extent of reductions of stomatal conductance. The photosynthesis : stomatal conductance ratio calculated prior to the excision in water and in the period of transient inhibition of photosynthesis showed no significant differences : 0.093 and 0.096 mmol · mol^{-1}, respectively. This agrees with results obtained with *Lycopersicon esculentum* but is in contrast to results obtained with *Helianthus annuus* (HEBER *et al.* 1986). Calculation of the intercellular CO_2 partial pressure (p_i) revealed almost constant values in the period of transient inhibition of photosynthesis, i.e. the carboxylation efficiency appeared to have decreased. But, according to previous results, the calculation of p_i may have led to erroneous results due to the non-uniform stomatal closure observed in *Vitis* species under stress conditions (DOWNTON *et al.* 1988, DÜRING 1992). In contrast to the experiments carried out with *Helianthus annuus* where the volume of water covering the petioles was > 10 ml (GSELL *et al.* 1989) we used water volumes of 3 ml to have a higher concentration of the presumed soluble inhibitor. However, even the addition of petiole pieces or extracts to the water filled tube, which induced inhibition of photosynthesis in *Helianthus*, did not intensify transient inhibition of photosynthesis in excised leaves of grapevines, while continous flushing of vine petioles with water during and after excision did not prevent this reaction. In *Helianthus* excision of petioles in water caused a stimulation of dark respiration; no such alterations were found in *Vitis*.

Conclusion

The rapid reaction of stomata after excision of petioles of droughted leaves provides further evidence for epidermal cells to change in water status without much change in water status of the bulk mesophyll. A direct water flux to the sites of transpiration appears to be a prerequisite to explain both, the rapid stomatal response to excision and the high rates of water substitution in transpiring vine leaves (SMART and COOMBE 1983: Tab. II). The details of bulk water flow and water diffusion in leaves are still unclear and need to be elucidated. It would also be of interest to examine if — like in *Pyrus* — leaves of *Vitis* have internal cuticles ‚lining the undersurfaces of epidermal cells and possibly coating surfaces of nearby mesophyll cells' (BOYER 1985). In contrast to *Helianthus annuus* and *Arbutus unedo* the rapid response of photosynthesis of *Vitis berlandieri* leaves to petiole excision in water is not mediated by the release and transport of soluble inhibitors of photosynthesis in the transpiration stream. Rather the reactions are the result of pressure changes within the xylem brought about by the free access of water to the xylem system. These pressure changes obviously run through the vine like hydraulic waves communicating between leaves independent of their position.

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References

BOYER, J.S; 1985: Water transport. Ann. Rev. Plant Physiol. **36**, 473-516.

DARWIN, F.; 1897: Observations on stomata by a new method. Proc. Cambridge Philos. Soc. **9**, 303-308.

DOWNTON, W.J.S.; LOVEYS, B.R.; GRANT, W.J.R.; 1988: Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. New Phytol. **110**, 503-509.

DÜRING, H.; 1988: CO_2 assimilation and photorespiration of grapevine leaves: Responses to light and drought. Vitis **27**, 199-208.

DÜRING, H.; 1992: Low air humidity causes non-uniform stomatal closure in heterobaric leaves of *Vitis* species. Vitis **31**, 1-7.

DÜRING, H.; LOVEYS, B.R.; 1982: Diurnal changes in water relations and abscisic acid in field-grown *Vitis vinifera* cvs. I. Leaf water potential components and leaf conductance under humid temperate and semiarid conditions. Vitis **21**, 223-232.

GSELL, W.; KIIRATS, O.; HARTUNG, W.; HEBER, U.; 1989: Inhibition of photosynthesis of sunflower leaves by an endogenous solute and interdependence of different photosynthetic reactions. Planta **177**, 367-376.

HEBER, U.; NEIMANIS, S; LANGE, O.L.; 1986: Stomatal aperture, photosynthesis and water fluxes in mesophyll cells as affected by the abscission of leaves. Simultaneous measurements of gas exchange, light scattering and chlorophyll fluorescence. Planta **167**, 554-562.

IWANOFF, L.; 1928: Zur Methodik der Transpirationsbestimmung am Standort. Ber. Dt. Bot. Ges. **46**, 306-310.

MANSFIELD, T.A.; DAVIES, W.J.; 1981: Stomata and stomatal mechanisms. In: L.G. PALEG and D. ASPINALL (Eds.): The Physiology and Biochemistry of Drought Resistance in Plants, 315-346. Academic Press, Sydney.

NONAMI, H.; SCHULZE, E.-D.; 1989: Cell water potential, osmotic potential, and turgor in the epidermis and mesophyll of transpiring leaves. Planta **177**, 35-46.

RASCHKE, K.; 1970: Stomatal responses to pressure changes and interruptions in the water supply of detached leaves of *Zea Mays* L. Plant Physiol. **45**, 415-423.

SCHOLANDER, P.F.; HAMMEL, H.T.; BRADSTREET, E.D.; HEMMINGSEN, E.A; 1965: Sap pressure in vascular plants. Science **148**, 339-346.

SHERIFF, D.W.; MEIDNER, H.; 1974: Water pathways in leaves of *Hedera helix* L. and *Tradescantia virginiana* L. J. Exp. Bot. **25**, 1147-1156.

SMART, R.E.; COOMBE, B.G.; 1983: Water relations of grapevines. In: T. T. Kozlowski (Ed.): Water deficits and plant growth. Vol. **VII**, 137-196. Academic Press, New York.

TYREE, M.T.; CHEUNG, Y.N.S; 1977: Resistance to water flow in *Fagus grandifolia* leaves. Can.J. Bot. **55**, 2591-2599.

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

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