Stomatal patchiness of field-grown Sultana leaves: Diurnal changes and light effects

by

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S u m m a r y : Unirrigated, field-grown Sultana vines were used to study diurnal changes of stomatal patchiness under the natural ambient conditions of South Australia. Non-homogenous aperture of stomata (patchiness) was determined by an infiltration technique and by counting infiltrated patches. Stomatal conductance was linearly related to the number of patches with open stomata ($r^2 = 0.622$). Both parameters increased in the early morning (maxima at 8 a.m.) and decreased thereafter. In the afternoon stomatal conductance and the number of patches with open stomata remained at a low level. When dark adapted leaves were exposed to sun light (900 -1000 μmol quanta $\cdot$ m^{-2} $\cdot$ s^{-1}) the number of patches with open stomata increased from 23 to 143 cm^{-2} within 15 min, indicating that besides several stress factors light induces non-homogenous stomatal opening. It is assumed that stomatal action of single patches is autonomous, i.e. stomata of single patches will regulate water loss and CO_2 uptake according to local changes of ambient and endogenous conditions.

K e y   w o r d s : stomata, stomatal conductance, patchiness, diurnal cycle, light, water stress.

Introduction

Since the pioneering work of Laisk et al. (1980) and Laisk (1983) indicating that stomatal aperture may not be homogenous over the entire leaf area numerous results have confirmed the existence of non-uniform gas exchange of leaves, also referred to as "patchiness". Using different techniques Downton et al. (1988 a, b, 1990) and Düring (1992) have demonstrated that under conditions of water stress, low air humidity, salinity or after application of abscisic acid (ABA) photosynthesis of Vitis leaves is restricted to small leaf areas. This results in a reduction in functional leaf area with proportional decreases in photosynthesis and stomatal conductance. Thus a reduced CO_2 fixation under these stress conditions can not be ascribed to non-stomatal inhibition of photosynthesis of grapevine leaves but is caused by non-uniform stomatal closure unless severe stress conditions are achieved (Kaiser 1987).

Terashima (1992) has pointed out that species showing non-uniform stomatal behaviour differ in their leaf anatomy from species having uniform stomatal reactions. Bundle sheathes and their extensions lacking intercellular airspaces, together with the adaxial and abaxial epidermis form airtight compartments. This kind of leaf anatomy which has been referred to as "heterobarisch" (heterobaric) (Neger 1918) has been confirmed for grapevines (Düring, unpubl.).

So far almost all experiments concerning non-uniform stomatal conductance have been carried out with glasshouse-grown plants under laboratory conditions. Only Downton et al. (1988 a) briefly mention the occurrence of non-uniform stomatal behaviour of outdoor-grown Riesling leaves.

In order to study stomatal behaviour under natural ambient conditions diurnal changes of stomatal conductance and patchiness of Sultana leaves were determined under the arid conditions of South Australia. It will be shown that stomatal patchiness of waterstressed leaves varies significantly during the day and that light exposure after a dark period induces non-uniform stomatal opening.

Material and methods

P l a n t s : At the Division of Horticulture (CSIRO) in Adelaide, Australia, 10-year-old ungrafted, field-grown Sultana vines were irrigated at 4-week intervals during the vegetation period. Diurnal changes of stomatal behaviour were determined on December 2nd and 3rd at the end of a non-irrigation period without rainfall. All measurements were made on fully illuminated leaves.

S t o m a t a l   c o n d u c t a n c e   a n d   v i s u a l i -
s i n g   p a t c h i n e s s : Fully expanded but not senescent leaves at the outer canopy were prepared by trimming the leaf area to rectangles symmetrical about the midrib (ca. 3.5 cm x 6 cm) using a pair of scissors. After measuring stomatal conductance (Delta-T, Cambridge, UK) at 6 locations on the left and right hand side near the central vein the leaf blade was inserted into a plastic syringe (100 ml) the upper end of which had been cut off. The open end of the syringe, filled with 50 ml water, was sealed by a rubber seal which enclosed the petiole (Fig. 1). This construction enabled us to infiltrate water into the blades of attached leaves. In order to eliminate the remaining air from the leaf surface and from substomatal cavities via open or partly open stomata the piston was pulled outward to
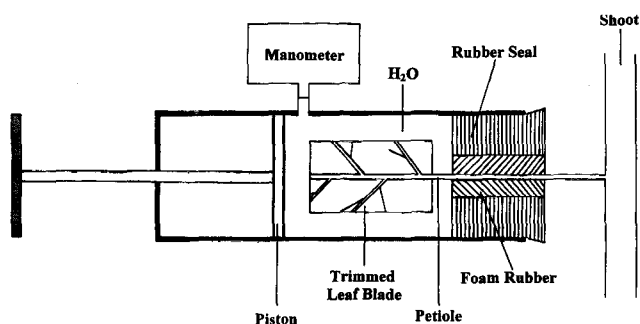
Fig. 1: *In situ* infiltration of water into leaves by a modified plastic syringe

produce a partial vacuum (Digital Pressure Meter, DPM 35, Wallace and Tiernan, Günzburg, Germany, connected to the syringe). Knocking the syringe led to an accumulation of the air bubbles in the upper part of the syringe; this air was removed from the syringe by partly opening the rubber seal. After closing the syringe again the water column in the syringe was set under pressure (0.15 MPa); thus water penetrated into the leaf blade via the open stomatal pores. Subsequently the leaf blade was removed from the syringe, blotted dry and immediately photographed (black and white film) together with a scale either in full sunlight or using 2 spot lights. Dark green patches under open stomata could clearly be distinguished from lightgreen patches with closed stomata. Photographing on a light bank (backlight) as has been described in an earlier paper for thin Müller-Thurgau leaves (DÜRING 1992) was less suitable for thick Sultana or Cabernet Sauvignon leaves. Single patches with open stomata were counted per cm^2 leaf area from the photographic prints.

Results

On December, 2nd and 3rd diurnal changes of stomatal conductance were determined using leaves of field-grown Sultana vines. These days were sunny and dry (air humidity 24-27 %), leaf temperature rising to a maximum of 37 ° C in the early afternoon. The unirrigated Sultana vines had a predawn leaf water potential of -0.44 MPa; after sunrise the leaf water potential decreased to a minimum value of -1.45 MPa at 10.30 a.m. and thereafter increased again to -1.06 MPa at 4 p.m. Stomatal conductance increased rapidly from very low levels at sunrise and was highest in the early morning at ca. 8 a.m., thereafter the values dropped rapidly to reach a minimum of ca. 16 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$ between 2.30 and 5 p.m.. The number of patches with open stomata increased after sunrise from less than 20 to a maximum value of ca. 155 patches per cm^2 at ca. 8 a.m. and decreased in the afternoon to the low level observed at sunrise (Fig. 2 a-d). Stomatal conductance was linearly related to the number of patches with open stomata (Fig. 3). It appears that under field conditions stomata of individual patches react independently to ambient and/or endogenous changes. Patches with open stomata are either almost homogenously distributed (Fig. 2 b) or they accumulate in certain leaf
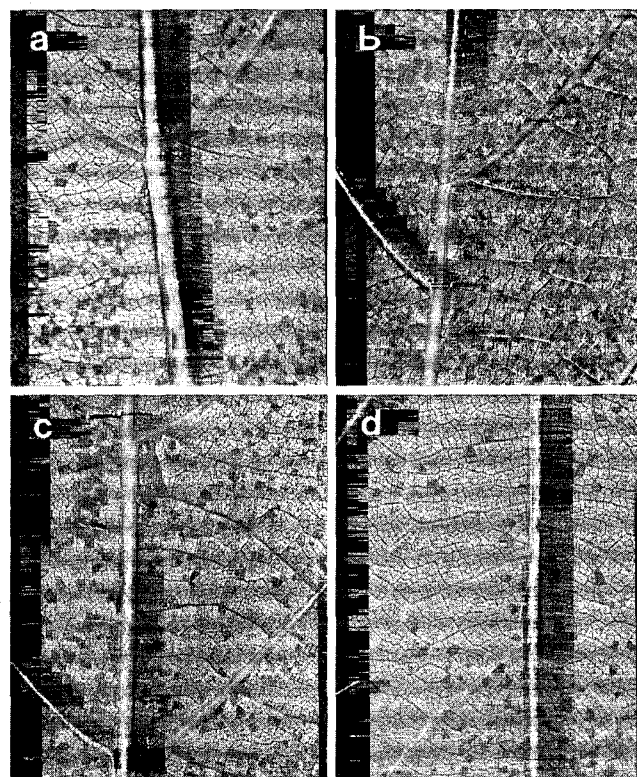

Fig. 2: Diurnal changes of patchiness at the abaxial epidermis of Sultana leaves. Dark spots indicate patches with open stomata. **a**: at 7 a.m., **b**: at 10 a.m., **c**: at 2 p.m., **d**: at 5 p.m.
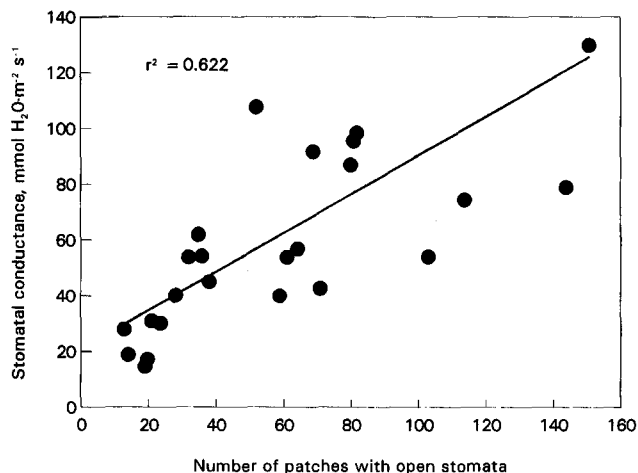

Fig. 3: Stomatal conductance vs. number of patches with open stomata (n = 24).

areas (Fig. 2 a, c). Thus it was impossible to relate the position of patches to the leaf topography, e.g. to its vein system.

These results and previous investigations have shown that the increase of stomatal conductance in the early morning is mainly due to the increase of light intensity (e.g. DÜRING and LOVEYS 1982). To study the effect of a sudden exposure of predarkened leaves to full sun light leaves of field-grown Sultana vines were cut from the vine and placed with their petioles in water. After cutting the petioles again under water the leaves were kept in the dark for 2 h to induce stomatal closure. Immediately after exposure to sunlight (900-1000 μmol quanta $\cdot m^{-2} \cdot s^{-1}$) and

5, 10 or 15 min thereafter water was infiltrated into the leaves. As is shown in Fig. 4 a-d stomata do not open homogenously after exposure to sun light; instead, infiltrated patches can be distinguished from non-infiltrated patches.
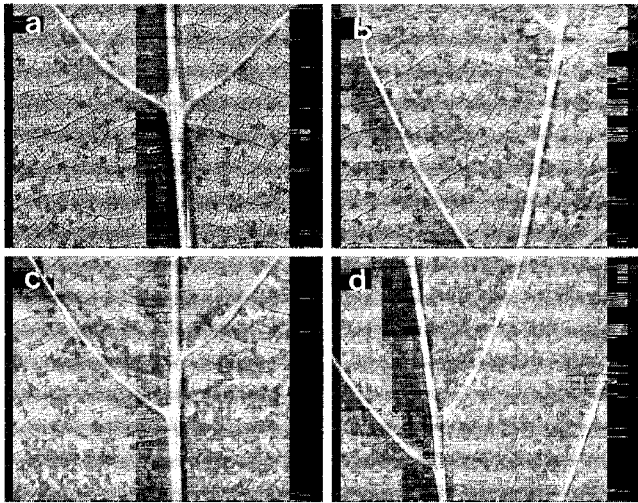

Fig. 4: Light induced patchiness of Sultana leaves. **a**: 0.5 min, **b**: 5 min, **c**: 10 min, **d**: 15 min after exposure of dark-adapted leaves to sun light (900-1000 μmol quanta $\cdot$ m^{-2} $\cdot$ s^{-1}) (details: Fig. 2).

Discussion

The infiltration technique presented in this paper has several advantages: (1) Stomatal patches with open stomata can be clearly distinguished from those with closed stomata, (2) the method is non-destructive and infiltrations can be repeated on the same leaf blade, (3) it can be used *in situ* under field conditions, (4) the equipment is fairly cheap and easy to handle. Counting of single patches with open stomata was possible in the lower range of stomatal conductance, i.e. <150 mmol H$_2$O m^{-2} s^{-1}. At higher conductances the number of patches with open stomata was too high to differentiate between single patches; here a determination of the infiltrated water by weighing is preferred (DÜRING 1992).

WISE et al. (1992) and TERASHIMA (1992) have raised the question whether patchy stomatal behaviour plays a role in plants in their natural environment. It appears that patchiness is a common phenomenon of heterobaric leaves under natural ambient conditions. As in the field the light, temperature and air humidity conditions are expected to be different at various parts of a leaf blade, e.g. within a canopy, and to change with time, the heterobaric leaf structure might be a very efficient tool to perform a localized adaptation to changing climatic conditions. BEYSCHLAG and PFANZ (1990) and BEYSCHLAG et al. (1992) simulated a natural mediterranean summer in a growth chamber and found that *Arbutus unedo* L. and *Quercus suber* L. leaves were fully infiltratable in the early morning and in the late afternoon while during the periods before and after midday leaves showed a distinct patchiness. This corresponds to the often observed midday depression with a recovery of transpiration and photosynthesis in the late afternoon. In our experiments stomatal conductance and the number of patches with open

stomata were also high in the morning but did not recover in the afternoon (Fig. 2 e).

The reasons for low stomatal conductance at relatively high water potential of grapevine leaves in the afternoon have been examined earlier, e.g. by LOVEYS (1984), CHAVES et al. (1987) and CORREIA et al. (1990). Besides a higher sensitivity of stomata to the intercellular CO$_2$ partial pressure observed in the afternoon the possibility of the afternoon depression being associated with localized areas of water deficit within the leaf which might not affect the bulk leaf water potential has been discussed by CORREIA et al. (1990). In fact, this assumption is strengthened by the observation that bulk leaf ABA often rises in late afternoon despite a rise in bulk leaf water potential at this time (LOVEYS and DÜRING 1984). As this ABA increase is unlikely to be result of an ABA import from xylem (see CORREIA et al. 1995) a local synthesis of ABA might be expected. Open stomata of single patches may induce localized water deficits and increases of the ABA level which in turn may induce localized stomatal closure. This view is supported by our excised leaf experiment; presumably the bulk leaf water potential was close to zero in these leaves and yet they were shown to be patchy a few minutes after exposure to light. All these results indicate that bulk leaf water potential is not controlling patchiness but ABA may be. Work is in hand, to show that patchiness is a dynamic phenomenon, i.e. patches with open stomata may turn into patches with closed stomata within rather a short time (CARDON et al. 1994, DÜRING and STOLL in prep.).

Conclusion

As a consequence of non-homogenous stomatal conductance and photosynthesis it is recommended to measure gas exchange of grapevine leaves using relative large leaf areas which include both, patches with open and those with closed stomata; measurements at leaf areas of less than 1 cm^2 are usually associated with a high variability of gas exchange rates, thus 5 to 10 replicates at different locations of a leaf have to be performed. The technique presented in this paper can easily be used in the field; it may also be used by growers to make a decision whether grapevines have to be irrigated or not. A series of photos may serve to identify different stages of drought stress.

Acknowledgements

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