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Stomatal patchiness of grapevine leaves. II. Uncoordinated and coordinated stomatal movements

by

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S u m m a r y : The dynamics of stomatal patchiness of grapevine leaves (var. Richter 110) were studied by *in situ* infiltration of water into the intercellular spaces (see: Düring and Loveys 1996). As infiltrations were shown not to affect stomatal conductance (g) a series of experiments was performed in which a leaf segment was infiltrated and photographed repeatedly. While stomata of some patches did not alter their apertures within a 90-minute experiment, others opened and closed their stomata more or less frequently leading to irregular fluctuations of patches with open, partly open and closed stomata. In contrast to this uncoordinated behavior coordinated, synchronous stomatal movements were recorded by gas exchange. Sinus wave-like stomatal oscillations with periods of 32–70 min and amplitudes of 38–95 mmol CO_2 m^{-2} s^{-1} at constant ambient conditions were observed in a 12 h experiment. The stomatal oscillations were closely related to rhythmic alterations of the intercellular CO_2 concentration (c_i) and to the rate of CO_2 assimilation (A). An increase of amplitudes of g was associated with a decrease of the carboxylation efficiency (A/c_i) and the water use efficiency (A/g). It is concluded that uncoordinated, patchy fluctuations of stomatal apertures enable effective adaptation of single patches to changes of ambient stress factors.

K e y w o r d s : stomatal patchiness, stomatal conductance, stomatal oscillation.

Introduction

The simultaneous occurrence of different stomatal apertures („patchiness") over a leaf blade of grapevines has been demonstrated by autoradiography and infiltration techniques under laboratory and field conditions (Downton *et al.* 1988 a, b; Düring and Loveys 1996). Stomatal patchiness was shown to be associated with the heterobaric leaf type, i.e. it occurs preferably in leaves which are divided into laterally separated gas-tight airspaces by vessels with extensions of their bundle sheaths to the epidermes (Düring and Stoll 1996). Several stimuli have been shown to induce heterogenous stomatal aperture in leaves of grapevines, e.g. changes of water supply, air humidity, light intensity, ambient CO_2 concentration or the application of abscisic acid (Downton *et al.* 1986 a, b; Düring 1992; Stoll unpubl.).

According to Raschke (1975) the stomatal control system is never in equilibrium except when stomata are closed. Periodic variations of stomatal conductance („oscillations") have been observed in many plants; they are assumed to be caused by a time delay between the interaction of different feedback loops (reviews: Barrs 1971; Raschke 1979). With respect to leaves showing non-uniform stomatal behaviour this implies that the spatial, non-random distribution of patches over a leaf is not static but changes with time. The aim of this paper was to study the dynamics of stomatal apertures of single patches *in situ*. It will be demonstrated that stomata of grapevine leaves can react in two ways: uncoordinated (non-synchronous movements) leading to a steady state of gas exchange or coordinated (synchronous movements) leading to stomatal oscillations of gas exchange.

Material and methods

Three-year-old potted grapevines, var. Richter 110, were cultivated under open air conditions, they were regularly supplied with mineral nutrients and water and protected against fungus diseases when necessary.

In situ infiltration of water into leaves was performed under glasshouse conditions as described earlier (Düring and Loveys 1996). Leaves were reduced in size by cutting a rectangle (2 cm x 4 cm) along a major vessel system. The remaining leaf was evacuated (-50 kPa for 1 s) and then distilled water (25 °C) was pressed into its intercellular spaces (70 kPa for 1 s) using a modified syringe. Immediately after drying the leaf blade by blotting-paper stomatal patchiness was recorded by photographing the lower leaf surface on a light bank (black and white film). Non-infiltrated areas appeared darker than infiltrated areas. During the experiment ambient temperature ranged from 28 to 30 °C, air leaf vapor pressure deficit was 20–21 $Pa \cdot kPa^{-1}$, and light intensity 700–900 µmol quanta m^{-2} s^{-1}.

Gas exchange parameters were always determined at constant climatic conditions within the cuvette (leaf temperature 24 °C, air leaf vapor pressure deficit 16 $Pa \cdot kPa^{-1}$, CO_2 concentration 350 ppm, light intensity at saturation 900 µmol quanta m^{-2} s^{-1}) using a „Miniküvetten-System" (Walz, Germany). In the experiment, in which oscillations were recorded ambient climatic conditions were kept constant except for room temperature (25–29 °C).

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Results and discussion

In situ infiltration: There is much evidence for the assumption that stomata are permanently adapting to endogenous and/or ambient changes (RASCHKE 1975). As a consequence photographs of patchy leaves obtained by infiltration will represent only a momentary status of stomatal patchiness which may change shortly after. To visualize alterations of patchiness over time we intended to repeat infiltration at the same part of a leaf several times and to compare the photographs obtained after infiltration. In preliminary tests we examined possible side-effects of repeated infiltrations of water into attached leaves. Leaf blades of Richter 110 vines were reduced in size and in a 60 min experiment infiltrated 10 times. After each infiltration stomatal conductance of the leaf blade was determined by gas exchange. No significant alterations of gas exchange due to infiltration were recorded; this was in accordance with the observation that at latest 3 min after infiltration dark, infiltrated patches were no longer to be detected. It remains open if the infiltrated water is evaporated via open stomata and/or if it is absorbed by the mesophyll.

If repeated infiltrations do not affect stomatal conductance this method should be suitable to study the expected alterations of patchiness over time. Within a 90 min experiment the defined area of a leaf attached to a potted vine was infiltrated every 10 min and photographed thereafter. The result is shown in Fig. 1, where white areas denote patches with open stomata, grey areas those with partly open stomata and black areas those with closed stomata. It appears that the stomata of some patches did not alter their apertures, e.g. patch 12, while others altered their apertures more or less frequently. The results shown in Fig. 1 demonstrate that stomata of single patches behave as individuals; they appear to be dynamic and uncoordinated. This behaviour is substantiated by investigations of SIEBKE and WEIS (1995) who were able to demonstrate that stomata of a single patch opened and closed concertedly, i.e. oscillated. Despite single patchy fluctuations the sum of water loss and CO_2 uptake of such a leaf per unit time may be fairly constant due to the heterogeneity and superposition of movements.

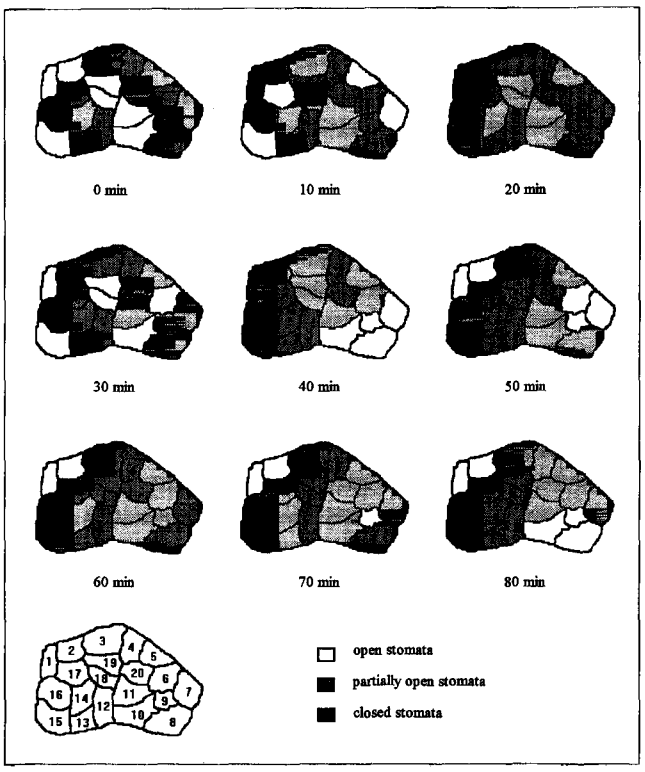

Fig. 1: Alterations of stomatal apertures of a group of patches in an *in situ* experiment.

Stomatal oscillations: It appears to be conceivable - under certain conditions - that the patchy fluctuations (Fig. 1) can be coordinated leading to synchronous stomatal movement of several patches. In fact, under the constant conditions of a climatized gas exchange cuvette we observed spontaneous extended stomatal oscillations.

Fig. 2 a indicates sinus wave-like oscillations with periods of 32–40 min and amplitudes of about 38 mmol CO_2 m^{-2} s^{-1} in the first 4 h (stage 1) and periods of 48–56 min and amplitudes of ca. 95 mmol CO_2 m^{-2} s^{-1} in stage 2. In other experiments the periods were longer, often 60–70 min (data not shown). This range of durations is quite common for stomata-related oscillations and must not be confused with photosynthetic oscillations with a period of one to a few minutes (COX 1968; BARRS 1971; WALKER *et al.* 1983; SIEBKE and WEIS 1995).

Fig. 2 b indicates that - obviously as a consequence of stomatal oscillations - the intercellular CO_2 concentration (c_i , calculated from gas exchange parameters) and, also, the rate of CO_2 assimilation (A) oscillate synchronously

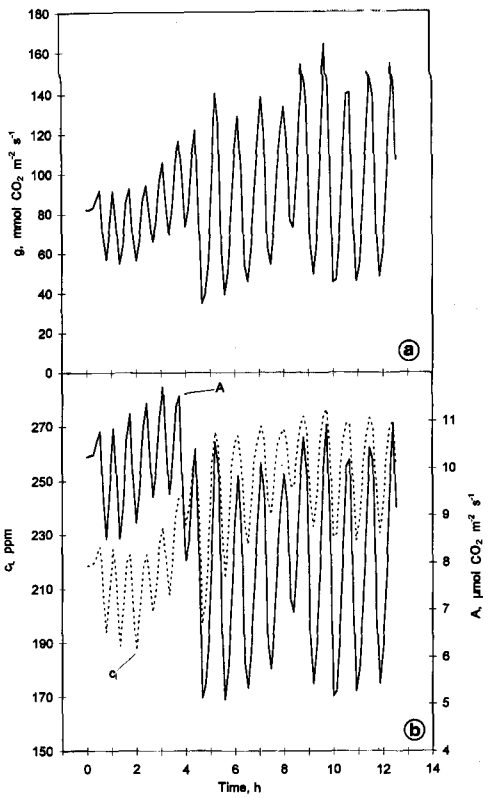

Fig. 2: Stomatal oscillations (g_{CO_2}) at constant ambient conditions of the cuvette, determined by (**a**) gas exchange measurements leading to (**b**) synchronous alterations of intercellular CO_2 concentration (c_i) and photosynthesis (A).

with stomata. In accordance with the difference of periods and amplitudes of stomatal conductance between stage 1 and 2 (Fig. 2 a) the periods and amplitudes of the c_i and A increase from stage 1 to stage 2. Altogether, there is a trend of c_i to increase and of A to decrease during the experiment. This trend indicating a decrease of the water use efficiency is also reflected in the A/g ratio where small amplitudes are associated with a higher A/g relationship (stage 1) and large amplitudes with a lower A/g ratio (stage 2) (Fig. 3).

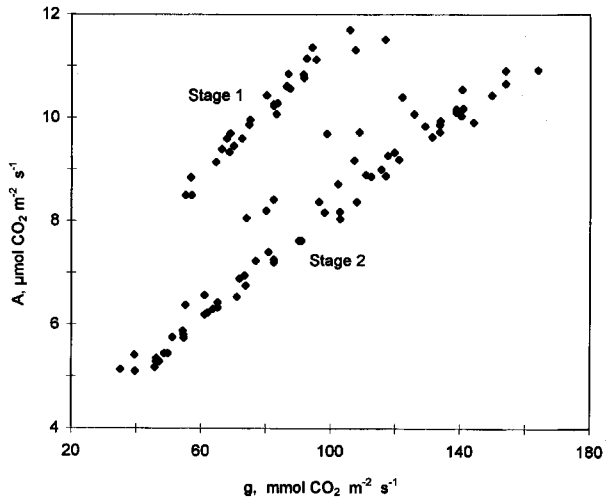

Fig. 3: Photosynthesis (A) to stomatal conductance (g) relationship. Note that the larger amplitudes of stomatal oscillation are associated with a lower A/g ratio.

It has to be stressed that the oscillations started spontaneously under constant ambient conditions of the cuvette, i.e. their origin is unkown. Moreover the causes of alterations of the amplitude remains obscure. All attempts to induce oscillations, e.g. by decreasing air humidity or altering ambient CO_2 concentration (CARDON *et al.* 1994) failed so far. Oscillations died out immediately when leaf petioles were cut under water (data not shown), indicating an association with changes of epidermal turgor.

From experiments with *Glechoma hederacea* using high resolution assimilation imaging SIEBKE and WEIS (1995) concluded that the starting point of large area oscillations detectable in gas exchange measurements could be the moment where preexisting oscillations of single patches are synchronized. It can be speculated that during coordinated stomatal movements of a huge number of patches physiological parameters like epidermal turgor, abscisic acid level and intercellular CO_2 concentration are equalized while under non-coordinated conditions these parameters differ from patch to patch. According to SIEBKE and WEIS (1995) stomatal oscillations will cause the chloroplasts to cycle between two extreme control states. E.g., at the minima of oscillations photosynthesis will be CO_2-limited and - at constant light intensity - the risk of photoinhibition is high. Thus, coordinated stomatal movements of heterobaric leaves appear to be less favorable with respect to an optimization of gas exchange parameters. As an exception to the rule they may die out sooner or later due to a loss of synchronization. On the other hand, uncoordinated stomatal apertures will protect most of the leaf area from becoming severely photoinhibited by periodically exchanging areas with open and closed stomata (BEYSCHLAG *et al.* 1994; POSPISILOVA and SANTRUCEK 1994). SCHEUERMANN *et al.* (1991) observed patchy behaviour predominantly in species like *Helianthus annuus*, which adopt photorespiration as a mechanism for dissipation of excess energy without loss of water. Thus from an ecophysiological point of view uncoordinated stomatal movements may enable small leaf units to adapt individually to localized changes of ambient stress factors in a very effective manner, e.g. to improve carbon gain and to reduce water loss under drought conditions.

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