Acta Oecologica 22 (2001) 161−173

S1146609X01011109/FLA

Towards a causal analysis of stomatal patchiness: the role of stomatal size variability and hydrological heterogeneity

Wolfram Beyschlag*, Jürgen Eckstein

Lehrstuhl für Experimentelle Ökologie und Ökosystembiologie, Universität Bielefeld - W4-107, Universitätsstr. 25, 33615 Bielefeld, Germany

Received 13 October 1999; revised 23 April 2001; accepted 25 May 2001

Abstract – The induction of the well known and widespread phenomenon 'stomatal patchiness' has been attributed to a variety of potential causes: from low PPFD levels, all kinds of stress conditions to CO_2-changes and even fungal infections. A mechanism which explains the occurrence of reproducible patterns of static (i.e. stable) stomatal patchiness at low PPFD levels is proposed. Further, experimental evidence is presented for the hypothesis that dynamic (i.e. not stable) stomatal patchiness is a consequence of heterogeneous water status in different parts of the leaf and can be induced by all ambient factors which cause such heterogeneities. © 2001 Éditions scientifiques et médicales Elsevier SAS

Acer platanoides **/ chlorophyll fluorescence / stomatal patchiness / stomatal regulation /** ***Xanthium strumarium***

1. INTRODUCTION

During the last decade, increasing evidence was found for the existence of pronounced ecophysiological variability at the single leaf level of plants The key phenomenon in this context is 'stomatal patchiness'. This expression was coined to describe the fact that the spatial distribution of stomatal aperture and, in consequence, photosynthetic activity on the leaf surface was in many cases found to be heterogeneous rather than homogeneous, as had been erroneously assumed for a long time (see [33, 58]). In between, stomatal patchiness has drawn the attention of many plant ecophysiologists and, from the considerable number of existing publications on the subject, it can be concluded that the occurrence of stomatal and photosynthetic heterogeneity seems to be a rather widespread phenomenon among vascular plants (for review, see [2, 44, 45, 58]). Since the occurrence of stomatal patchiness can lead to erroneous gas-exchange parameters when calculated under the assumption of stomatal homogeneity, the need for investigation of the mechanisms which cause this phenomenon is obvious. Factors identified to induce stomatal patchiness include water and salt stress [11, 12, 20, 21, 25, 52, 54], low air humidity [4, 5, 13, 34], increases in xylem ABA [10, 33, 50, 59, 60], cold stress [42], changes in light intensity [6, 14, 17, 23], toxic gases such as O_3 and SO_2 [19, 38, 39], changes in ambient CO_2 partial pressure [57, 63], light stress [55] and even fungal infections [55]. The majority of the above list represents stress factors, which normally lead to a stomatal closure. Changes in light intensity and in ambient CO_2 are the only factors which can induce stomatal patchiness in unstressed leaves.

At first, stomatal patchiness could only be detected and visualized statically by destructive methods, such as pressure infiltration of liquids into the leaves via the stomata (e.g. [3]), or indirectly through assimilation of radioactively labelled CO_2 (e.g. [10, 11]). An important step towards the causal analysis of the underlying mechanisms was the development of non-destructive chlorophyll fluorescence imaging techniques [8, 41, 50] which created the possibility to also study the spatial and temporal dynamics of the phenomenon. Similar to the radioactive labelling technique, this method analyses the heterogeneity of photosynthetic activity on the leaf blade. Photosynthesis is low and fluorescence is high in areas with low stomatal con

*Correspondence and reprints: fax: +49 521 1066038.
E-mail address: w.beyschlag@biologie.uni-bielefeld.de (W. Beyschlag).

ductance and vice versa. The method produces particularly clear images in heterobaric leaves, which due to their anatomy show no lateral CO_2 diffusion between neighbouring areoles. The most important result which was found by using this method was, that under water stress conditions, the distribution of areas with open and closed stomata was highly dynamic across the leaf surface as well as over time (e.g. [6, 16, 33]). In contrast, if unstressed plants and low PPFD values were used for induction of stomatal patchiness, Eckstein et al. [17] reported surprisingly stable temporal and spatial aperture distribution patterns.

Knowing that there is a pronounced size variability among the stomata on a particular leaf (e.g. [29]), it seems difficult to explain the phenomenon that groups of stomata (in heterobaric leaves, typically all stomata within an areole) close or open in a co-ordinated manner and that areas with open and closed stomata are located right next to each other in an almost bimodal distribution. A possible explanation can be found in a recent publication of Haefner et al. [26] where they present a spatially explicit model which combines the accepted physiological mechanisms of stomata regulation with the hydraulic interactions between neighbouring stomata. One of the basic assumptions in this model is that all stomata within a particular areole are hydraulically connected through the epidermal turgor in a way that aperture changes of single stomata propagate across the entire areole (for a more detailed representation see the Discussion below). Experimental results seem to confirm the respective model predictions [35].

In the present paper, we propose a mechanism which in connection with the assumptions of Haefner et al. [26] explains the occurrence of reproducible static (i.e. stable) stomatal patchiness at low PPFD levels. Further, we provide experimental evidence for the hypothesis that dynamic stomatal patchiness is a consequence of heterogeneous water status in different parts of the leaf [58] and can be induced by all ambient factors which cause such heterogeneities.

2. MATERIALS AND METHODS

2.1. Plant material

Five-year-old plants of *Acer platanoides* were excavated and transferred into pots 4 months prior to the experiments. These potted plants were then grown under field conditions at an experimental field site near the University of Bielefeld. They were kept well watered and fertilized with half-strength Hoagland solution at irregular intervals.

Plants of *Xanthium strumarium* were grown from seed in pots in a growth chamber. Plants were illuminated 12 h per day with a light intensity approximately 400 μmol quanta·m^{-2}·s^{-1} PPFD. Air temperature was 20 °C during the day and 15 °C at night. Relative air humidity was approximately 75 %. Plants were watered daily and fertilized with half-strength Hoagland solution every second day.

2.2. Gas exchange measurements

Stomatal conductance (g_s) of the experimental leaves was measured with a commercially available gas exchange measurement system (Minicuvette System, Walz, Meß- und Regeltechnik, Effeltrich, Germany with cuvette GK 0235). All gas exchange measurements were conducted at ambient CO_2 partial pressure of 353 ± 4 ppm and under 21 % oxygen. During the experiment, the system continuously logged the measurement data at 20-s intervals.

2.3. Imaging of chlorophyll fluorescence

Images of chlorophyll fluorescence [8] of the leaves enclosed in the gas exchange cuvette were obtained with a frame grabber board (PCgrab-G2, Matrix Vision, Oppenweiler, Germany) and a CCD camera (KP-M1, Hitachi, Tokyo, Japan) protected with a high-pass filter (RG-665, Schott, Mainz, Germany). Actinic light was supplied with a cold light lamp (KL-1500, Schott, Wiesbaden, Germany) and filtered through a cold filter (KG-1, Schott, Mainz, Germany) and a low-pass edge filter (650-FL-7, Andover, Salem, NH, USA). Light for saturation pulses was applied by a halogen lamp with an electronic shutter (FL-650, LUT-Schölly, Denzlingen, Germany) and was filtered through a low-pass filter (10-SWF-650, Newport, Irvine, CA, USA). PPFD of the saturation pulses (duration approximately 500 ms) was above 4 000 μmol quanta·m^{-2}·s^{-1}. Leaves were dark-adapted for 10 min before the capture of the F_m image. The non-photochemical quenching coefficient (qN) was calculated from images of leaf fluorescence during a saturation pulse of the dark-adapted leaf (F_m) and images of leaf fluorescence during a saturation pulse in actinic light (F'_m) using the formula of Daley et al. [8]:

$$qN = (F_m - F'_m)/(0.8 \times F_m)$$

This coefficient has been demonstrated to be an indicator for g_s [61].

In this paper, we present qN images (*figure 2*) as well as F'_m images (*figures 4, 6, 8*). In qN images, dark patches represent areas with relatively wide open stomata while regions with low apertures appear as light areas. In contrast, F'_m images reveal regions with open stomata as dark and those with closed stomata as light areas.

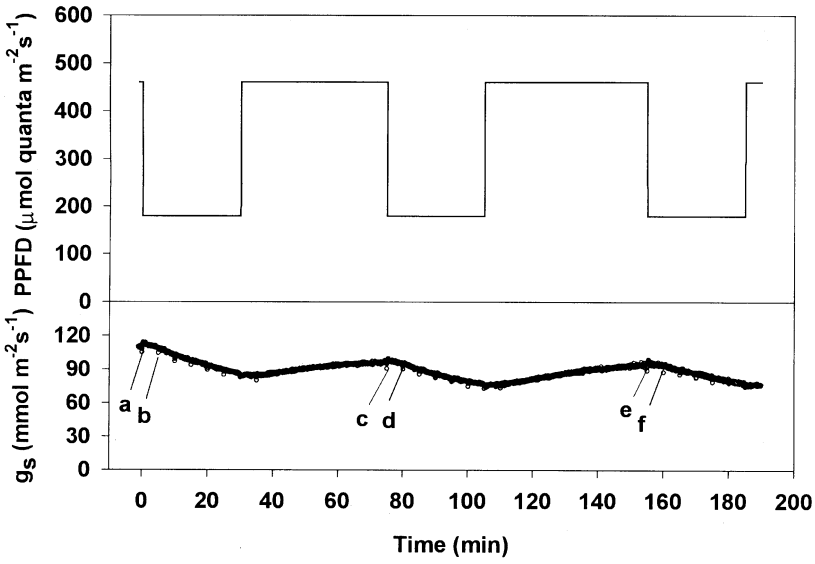

Figure 1. Experimental variations in incident PPFD level (top) and resulting changes in g_s (bottom) for an attached leaf of *A. platanoides* at $T_L = 25$ °C and $\Delta w = 15$ mPa·Pa^{-1}. The letters refer to the fluorescence images in *figure 2*.

2.4. Experiments

Each of the following experiments was carried out repeatedly with at least ten different leaves. Although the absolute values for g_s and the degree of patchiness varied considerably between individual leaves, the relative responses to the experimental treatments were rather similar.

2.4.1. Variation of PPFD

Intact leaves of *A. platanoides* were enclosed in the gas exchange cuvette at a leaf temperature (T_L) of 25 °C, a water saturation deficit of the air (Δw) of 15 mPa·Pa^{-1} and an intermediate light intensity of 450 μmol quanta·m^{-2}·s^{-1} PPFD until g_s reached an equilibrium. Subsequently the light intensity was rapidly decreased to 180 μmol quanta·m^{-}2·s^{-1} PPFD for 30 min. Then the light level was rapidly brought back to 450 μmol quanta·m^{-2}·s^{-1} PPFD. After equilibration of g_s, the treatment was repeated twice more (see *figure 1*).

2.4.2. Cutting of leaf veins

Intact leaves of *A. platanoides* were partially enclosed in the gas exchange cuvette at $T_L = 30$ °C, at an intermediate light intensity of 450 μmol quanta·m^{-2}·s^{-1} PPFD and a Δw of 30 mPa·Pa^{-1} in order to increase the transpirational water loss. After g_s reached an equilibrium a selected first order vein was dissected outside of the cuvette with a scalpel in order to create local water deficits on the leaf surface.

2.4.3. Infiltration of osmotics

Leaves of *A. platanoides* were cut and the cutting surface immediately transferred into a vessel with distilled water. Subsequently a 1-cm piece of the petiole was cut off under water. The leaf blade was then enclosed in the gas exchange cuvette at $T_L = 25$ °C, an intermediate light intensity of 450 μmol quanta·m^{-2}·s^{-1} PPFD and a Δw of 21 mPa·Pa^{-1}. After equilibration of g_s the distilled water was replaced by a mannitol solution of 300 mol·m^{-3}. This concentration represents an osmotic potential of 0.74 MPa and was chosen because Downton et al. [11] could induce pronounced stomatal patchiness with similar concentrated salt solutions.

2.4.4. Pressure application on the root system

Young potted plants of *X. strumarium* were grown in a such a way that the shoot grew through the hole in the lid of a commercially available Scholander-type pressure chamber (Model 1000, PMS Instrument Co., Corvallis, OR, USA; see also [40]). After 2-months, the shoot had pneumatically sealed the hole via its secondary growth. Now the pot with a slightly water-stressed plant was placed in the pressure chamber and the lid was carefully closed. A leaf of the plant was enclosed in the gas exchange chamber at $T_L = 30$ °C, 150 μmol quanta·m^{-2}·s^{-1} PPFD and a Δw of 30 mPa·Pa^{-1}. These conditions have been found to be suitable for the induction of stomatal patchiness in previous experiments (see [17]). After equilibration of

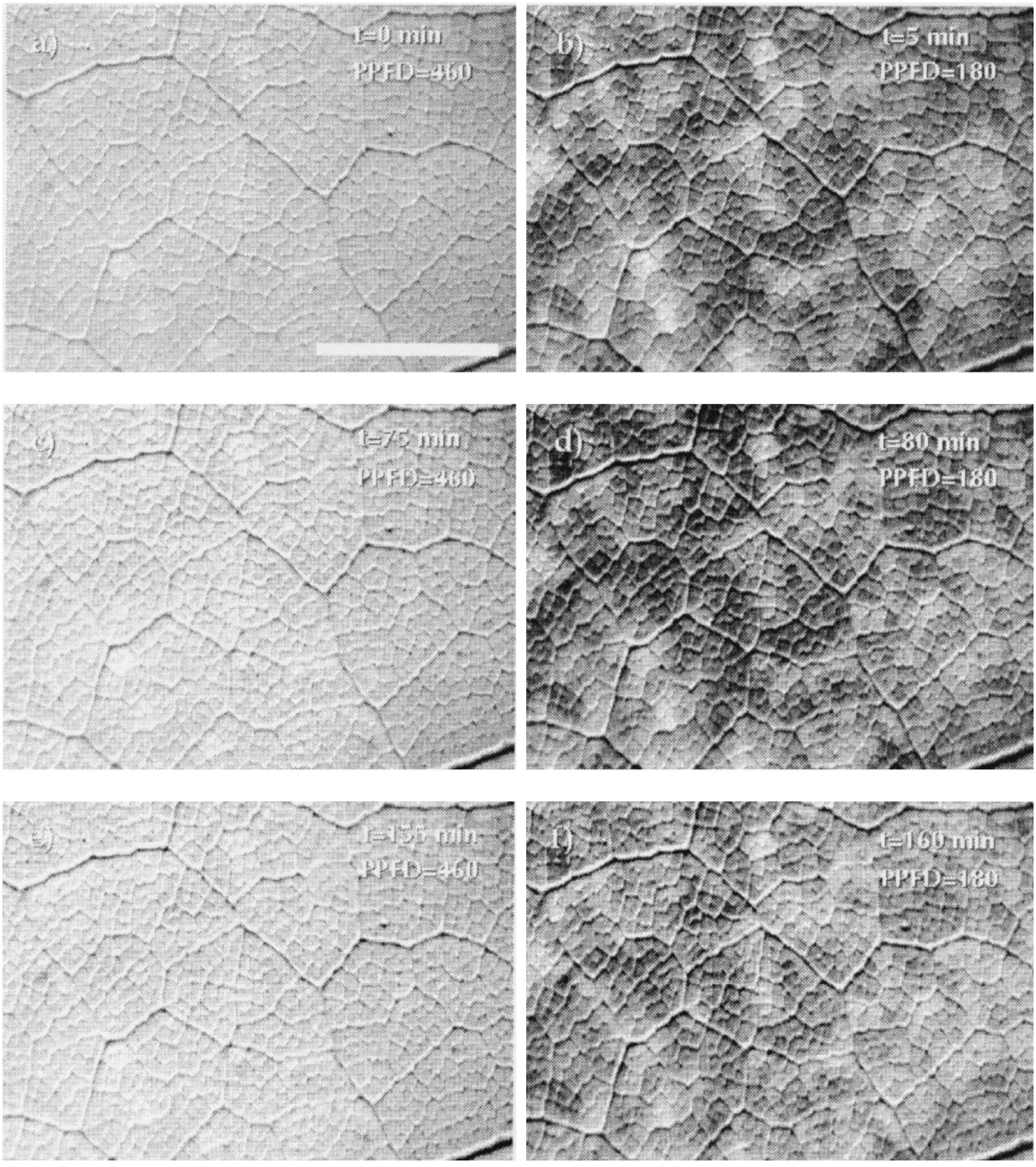

Figure 2. qN images from the experiment in *figure 1*. The white bar indicates 5 mm. a, c, e: qN at 450 μmol quanta·m^{-2}·s^{-1} PPFD; b, d, f: qN at 180 μmol quanta·m^{-2}·s^{-1}. The mean area fraction of dark areoles in panels b, d and f is 30.2 % (± 2.8 SD).

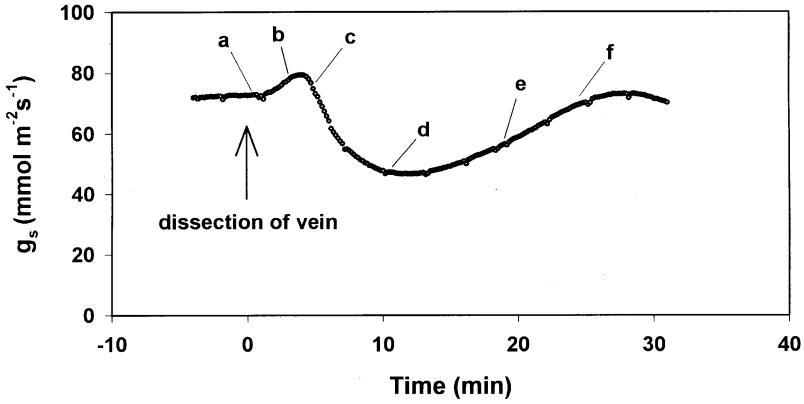

Figure 3. Time course of g_s of an attached leaf of *A. platanoides* after dissection of a major vein at time 0 (T_L = 30 °C; PPFD = 450 μmol quanta·m^{-2}·s^{-1}, Δw = 30 mPa·Pa^{-1}). The letters refer to the fluorescence images in *figure 4*.

g_s the hydrostatic pressure on the root system was quickly raised by 0.3 MPa. After 80 min, the pressure in the chamber was released to ambient values.

The points in time when the fluorescence images were taken during the various experiments is indicated in the respective figures.

3. RESULTS

3.1. Variation of PPFD

Changing incident PPFD between intermediate 450 and low 180 μmol quanta·m^{-2}·s^{-1} caused moderate but reversible changes in g_s of *A. platanoides* leaves (*figure 1*). The respective simultaneously recorded fluorescence (*figure 2*) shows a nearly homogeneous situation at all three 450-μmol quanta·m^{-2}·s^{-1} phases. However, after lowering the light intensity, a pronounced patchy fluorescence pattern developed almost immediately. Nearly identical spatial distribution patterns of dark and light areoles could be detected during all three low light phases when images taken at the same time distance after lowering the light were compared (*figure 2*: images b, d and f, all recorded 5 min after the light change).

3.2. Cutting of leaf veins

As shown in *figure 3*, the dissection of a major leaf vein of a well watered *A. platanoides* leaf led at first to an increase in g_s (the classical 'Iwanoff effect'). Subsequently a rapid and considerable decrease in g_s could be observed, indicating water stress in those portions of the leaf which were previously supplied by the dissected vein. However, about 15 min later, g_s increased again to values similar to those before dissection. After this, g_s began to oscillate (not shown). Similar effects have been reported for cut leaves [1, 41]. Obviously the water stress for the affected part of the leaf lasted only for a relative short time interval. After this the supply of the respective region is taken over by other still intact veins, a well known phenomenon which has already been described by Wylie [62]. The time course of fluorescence images of the affected leaf region shown in *figure 4* reflects these reactions. The major vein in the picture is the dissected one. Before and shortly after the dissection, chlorophyll fluorescence was homogeneous all over the leaf. After 5 min, the fluorescence became increasingly patchy. Maximum patchiness was observed 12 min after dissection. Subsequently and parallel to the increase in g_s, the patchiness disappeared and chlorophyll fluorescence became homogeneous again.

3.3. Infiltration of osmotics

The g_s data in *figure 5* show the typical stomatal reaction of an *A. platanoides* leaf to an increase of xylem potential [49]. Following a transient opening, stomata closed at first rapidly and later (after a small oscillation) more slowly. At the end of the experiment, g_s reached values close to zero. Looking at the time course of fluorescence images in *figure 6*, one can see at first the stomatal opening (less dark areas in panel b than in panel a). As soon as g_s declined, the fluorescence image became increasingly patchy and the area of dark (= closed) areoles increased considerably. At the end of the experiment the stomata on the leaf surface were almost homogeneously closed. Areoles with open stomata could only be detected in the near vicinity of major veins.

3.4. Pressure application on the root system

According to physical laws, pressure changes in one part of a system of liquid filled connected vessels should propagate almost immediately through the entire system. An increase of hydrostatic pressure on

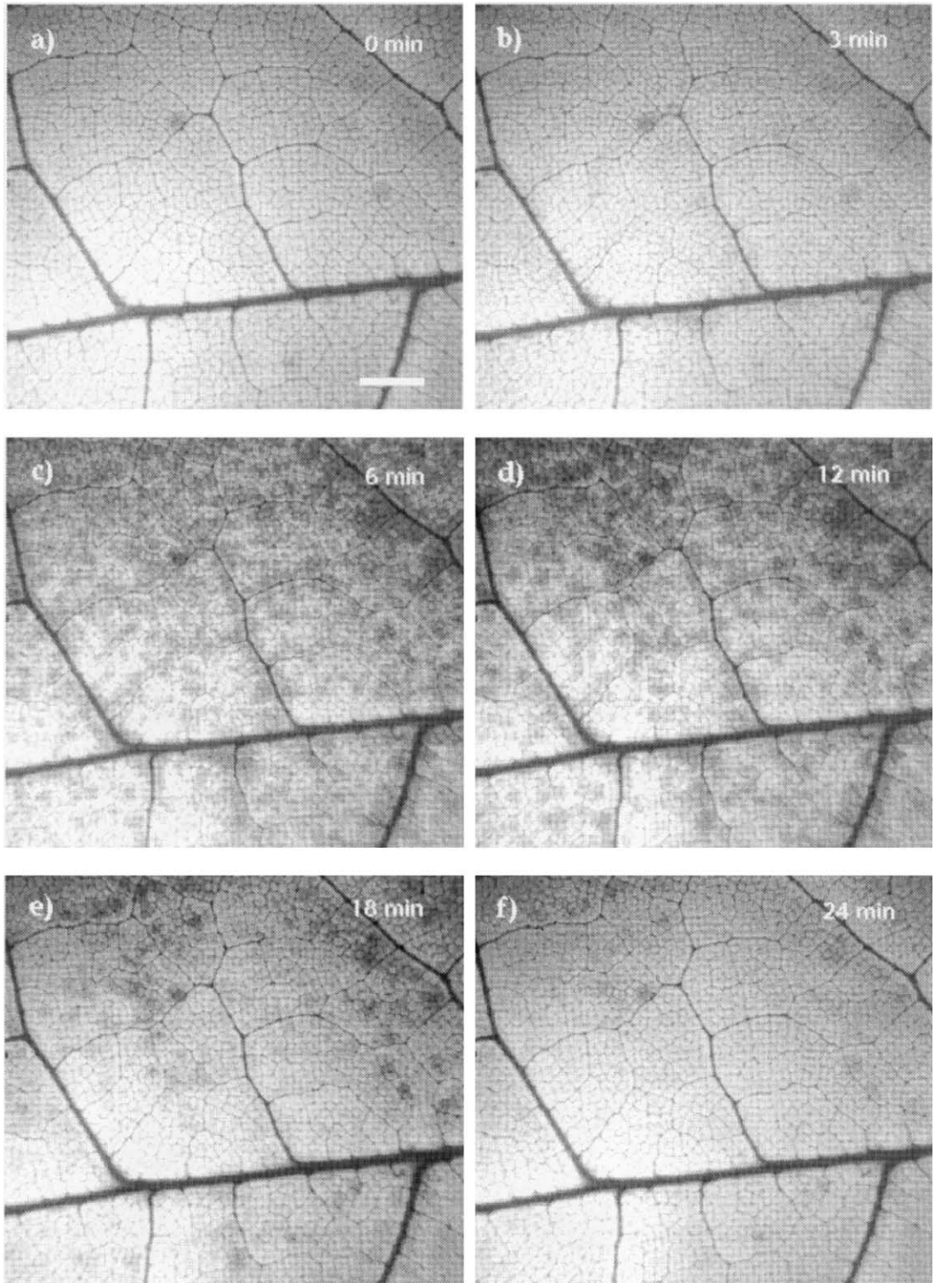

Figure 4. F'm images from the experiment in *figure 3*. The white bar indicates 5 mm.

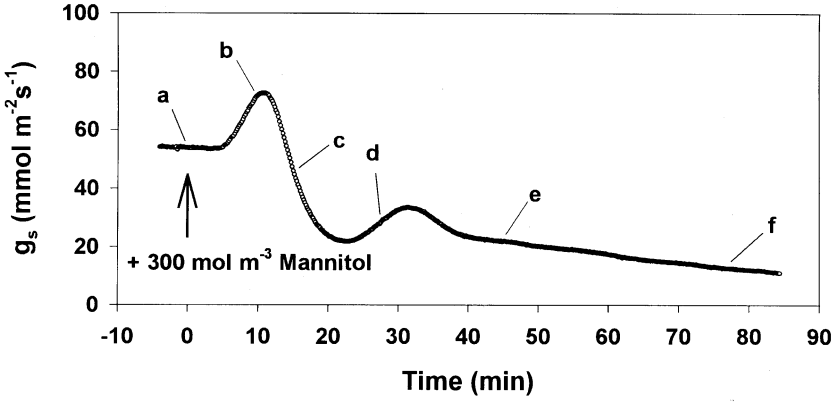

Figure 5. Time course of g_s of a cut leaf of *A. platanoides* after infiltration of 300 mol·m^{-3} mannitol via the xylem (T_L = 25 °C; PPFD = 450 μmol quanta·m^{-2}·s^{-1}, Δw = 21 mPa·Pa^{-1}). The letters refer to the fluorescence images in *figure 6*.

the root system should lead to an almost simultaneous turgor increase in all xylem vessels and, therefore, improve the water supply of all aboveground plant parts. Leaves of *X. strumarium* showed almost no reaction to this procedure. The g_s values decreased slightly (*figure 7*) and the fluorescence remained homogeneous all over the leaf (*figure 8*). Upon release of the pressure on the root system, g_s shows a short transient increase, indicating that the epidermis turgor decreased faster than the turgor of the hydropneumatically isolated guard cells (see also [56]). While some authors report marked changes in g_s in response to pressure changes [22, 46, 47, 51], others describe only minor effects similar to the present measurements [24, 53]. There was almost no time lag between the pressure changes and the respective responses of g_s. Due to the fact that all parts of the experimental leaf are almost simultaneously affected by these turgor changes, formation of major stomatal heterogeneity was not to expect and did not occur (*figure 8*).

4. DISCUSSION

The results of the present study provide experimental evidence that the occurrence of stomatal patchiness can be mainly explained through the occurrence of water status heterogeneity in different parts of the leaf. In order to better understand the considerations leading to this statement, the following theoretical considerations which include parts of the hypotheses presented by Haefner et al. [26] are necessary. One key feature for stomatal patchiness is the existence of a random variability of size among the stomata on a given leaf surface. This has been shown to be typical for the leaves of many angiosperms including the genus *Acer* (e.g. [15, 16, 29, 43]). After a leaf is fully differentiated this variability is fixed, i.e. in each areole there is a fixed size distribution of stomata. During stomatal closure, smaller stomata will reach limiting apertures for transpirational water loss earlier than larger stomata. The co-ordinated behaviour among all the different stomata within the same areole is brought about by an hydropneumatical coupling between neighbouring stomata via the epidermal turgor. As soon as during stomatal closure the first stoma reaches an aperture which markedly reduces water loss through its pore, the epidermal turgor in its surroundings increases relative to the guard cell turgor of the neighbouring stomata and thus forces them to close as well. (The 'mechanical advantage of the epidermis' [9] relative to the guard cells plays a key role in this connection.) This effect propagates across the entire areole until all stomata are homogeneously closed. Thus, under the effect of a closing factor which affects the entire leaf homogeneously, those areoles which have a relatively high fraction of smaller stomata will always be the first with homogeneously closed stomata. Assuming that the available amount of water for this particular leaf does not change, a reduction of transpirational water loss in the 'closed' areoles will increase the water availability for the still 'open' areoles which in consequence enables the stomata there to remain open. Since the size distribution of stomata on the leaf surface is fixed, the same closing factor should always produce identical distributions of open and closed areoles, as long as the water status is homogeneous throughout the entire leaf. This phenomenon has been called 'static stomatal patchiness' [26]. We experimentally simulated this situation by using

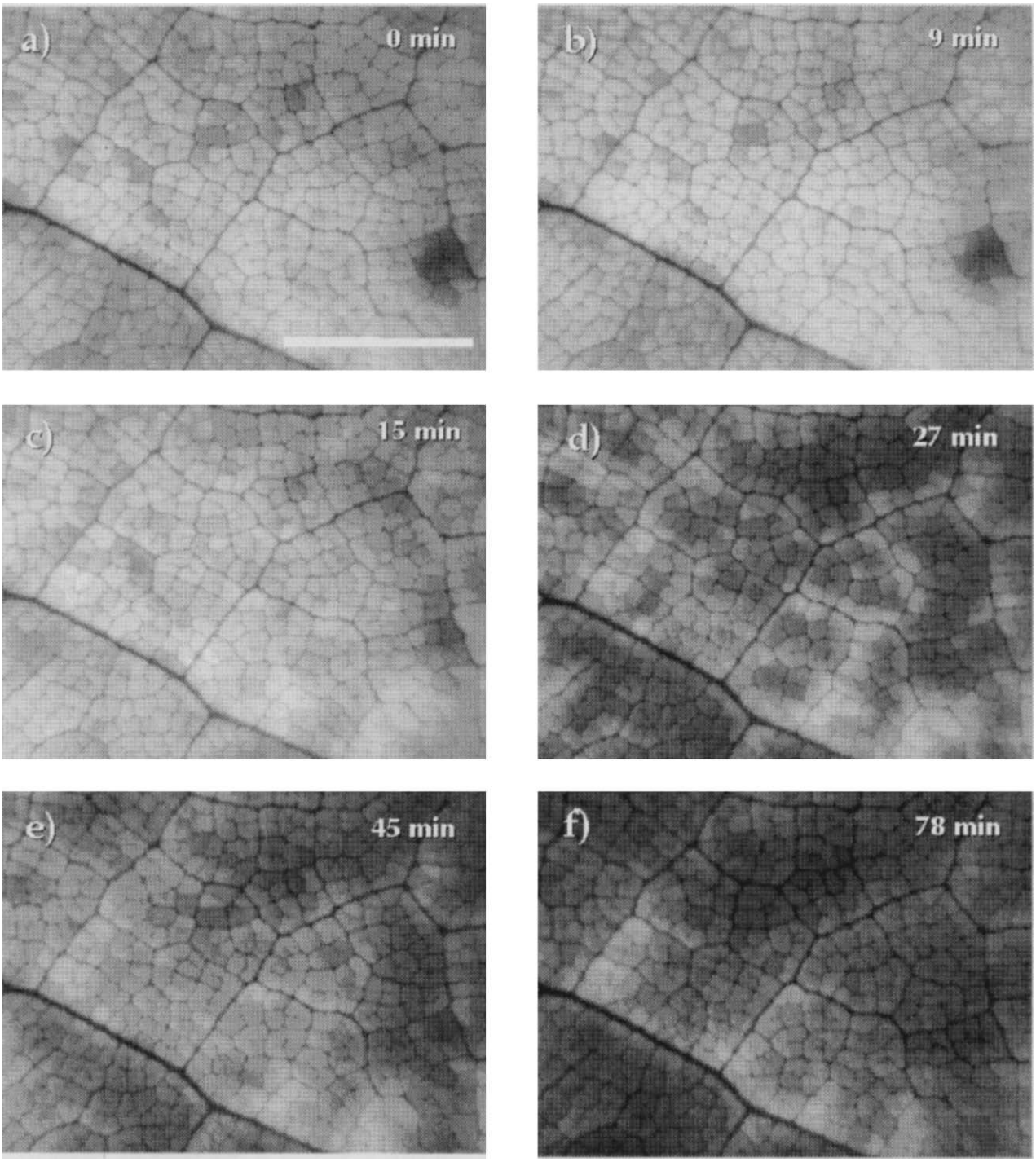

Figure 6. F'm images from the experiment in *figure 5*. The white bar indicates 5 mm.

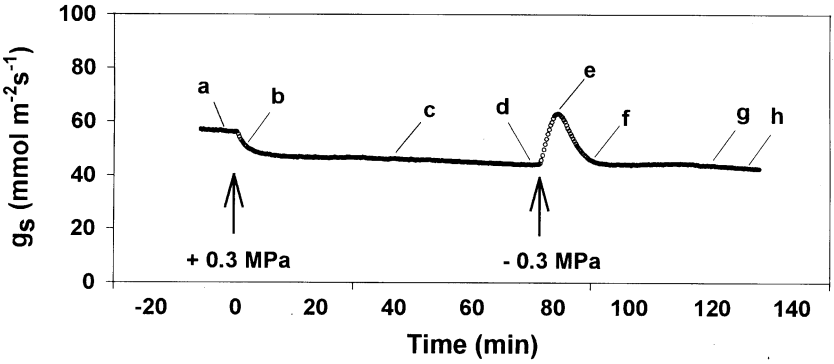

Figure 7. Reactions of g_s of an attached leaf of of *X. strumarium* to experimental manipulation of the hydrostatic pressure on the root system of the plant (T_L = 30 °C; PPFD = 150 μmol quanta·m^{-2}·s^{-1}, Δw = 30 mPa·Pa^{-1}). The letters refer to the fluorescence images in *figure 8*

low PPFD as a closing factor for unstressed leaves and in fact obtained the expected reproducible distribution of 'open' and 'closed' areas (*figure 2b, d, f*).

It has been shown that the epidermis is in close hydraulic contact with the evaporating sites of the mesophyll [36]. Thus, any change in the overall water status of a leaf will cause a change in epidermal turgor. Since the water status of the epidermis is a crucial parameter for the above described mechanism, any major change in the overall water status may potentially cause stomatal patchiness. In fact, a large number of studies describes a close connection between water stress effects and the occurrence of stomatal patchiness (for review, see [2]). However, the negative results of the pressure experiment (*figure 8*), where the water status of the entire leaf was changed quickly and almost simultaneously throughout the entire leaf, indicate that a second condition has also to be met. Stomatal patchiness can obviously only occur if the changes in water status create a spatial heterogeneity of epidermal turgor on the leaf surface. The present results (dissection of a major vein, infiltration of an osmotic; *figures 4, 6*) which induced gradual water status changes confirm this statement. Thus, the early hypothesis of Terashima [58] who presumed that the cause for stomatal patchiness might be a heterogeneous water status in different parts of the leaf seems to hold. Another prominent hypothesis, that heterogeneous ABA distributions within the leaf would cause stomatal patchiness (e.g. [31]) has already been rejected by the work of Eckstein et al. [18]. Thus, any external factor which has the potential to induce spatial heterogeneity in epidermal turgor should elicit stomatal patchiness.

Manipulations which result in heterogeneously distributed temporal changes of leaf water status lead to dynamic (i.e. not stable) stomatal patchiness (*figures 4, 6*). Similar dynamic effects have been observed after perturbations of the stomatal control which induced oscillations of g_s [6, 17, 57]. These oscillations were also visible in the distribution of 'open' and 'closed' areoles in the respective images of chlorophyll fluorescence. Under persisting water stress conditions, the water supply for the leaves of a plant will gradually decrease and even the 'open' areoles will experience water shortage and close their stomata. In consequence, the heterogeneous distribution of 'open' and 'closed' areas will gradually disappear until all areoles have reached homogeneously low stomatal apertures (*figure 6f*). Similarly, if the water stress disappears, temporal stomatal patchiness will reoccur during the reopening phase of the stomata and then disappear as soon as all areoles are homogeneously 'open' (Eckstein, unpubl. results).

Stomatal patchiness has predominantly been observed in heterobaric leaves where the areoles are hydropneumatically separated from each other by leaf veins with bundle sheath extensions (e.g. [2, 59]). Since the above mechanism for co-ordinated stomatal behaviour does not require hydropneumatical separation of the areoles, stomatal heterogeneity should also be possible in homobaric species. Some observations of this kind have in fact been made [16, 17, 30, 41]. However, detection is rather difficult since the respective methods mostly record differences in photosynthetic activity where the contours between areas of different aperture status are blurred or even erased by the lateral gas diffusion. A recently published new method which employs thermography to detect spatial and temporal variation of g_s over the leaf surface [28] may be helpful in this connection. Theoretically, stomatal patchiness can occur in any plant leaf, but the

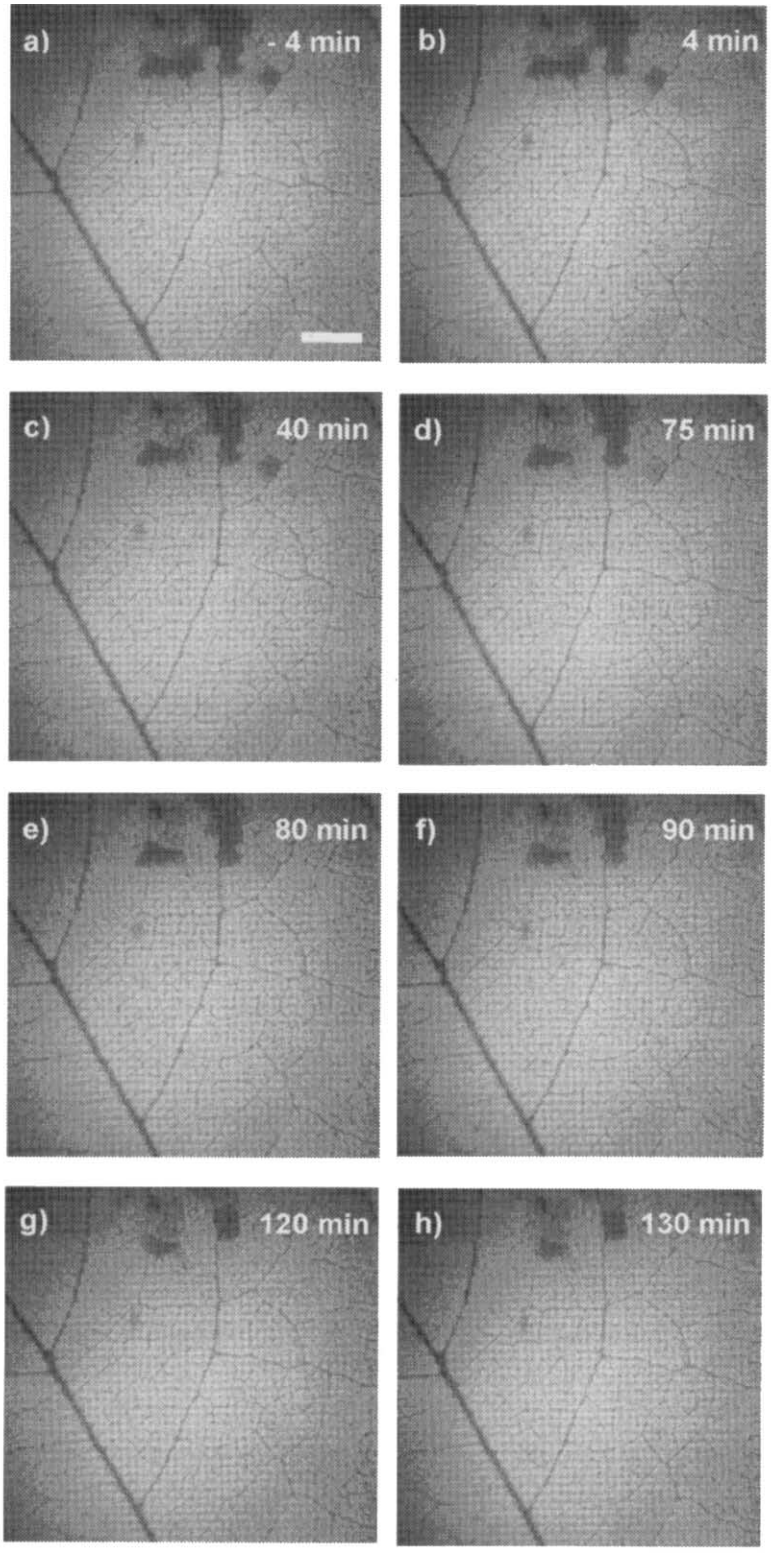

Figure 8. F'm images from the experiment in *figure 7*. The white bar indicates 5 mm.

probability is higher in heterobaric than in homobaric leaves [16].

Based on the above facts, it should be possible to predict the occurrence and magnitude of stomatal patchiness for leaves with a given water status and known ambient conditions. However, as anybody experimentally work on stomatal reactions knows, in spite of known deterministic single factor dependencies, stomatal reactions cannot always be predicted. Although the majority of replications of the present measurements revealed rather similar results, there was also a considerable number of experiments where completely unexpected stomatal reactions occurred. A highly probable explanation for this is, that the stomatal control mechanism is an ideal system for the development of chaotic behaviour. According to Nicolis and Prigogine [37], a complex system can become a 'dissipative chaotic system' if three conditions are met: (i) the system is an energetically open system and receives energy from outside (as all organisms do); (ii) it contains several feedback control mechanisms between its functional parts; and (iii) non-linear relationships exist between some system parameters. Condition (i) applies to all biological systems. Condition (ii) is also met by stomatal control (e.g. [7, 48]) and there are several non-linear relationships documented for the stomatal system (e.g. [7, 27, 32]). The increasing knowledge on the underlying mechanisms of stomatal patchiness will certainly help ecophysiologists (particularly those working in the field of leaf gas exchange) to minimize the risk for the occurrence of this phenomenon during particular experiments. However, due to the above facts, surprises will always be possible.

5. CONCLUSIONS

The occurrence of stomatal patchiness seems to be a by-product of stomatal adjustments of the leaf in response to changing environmental conditions rather than a biological meaningful phenomenon. The coordinated behaviour of all stomata within the same areole is brought about by a hydropneumatic coupling mechanism via the epidermal turgor [26]. In unstressed leaves, factors which induce stomatal closure lead to a rather stable and reproducable pattern of 'open' and 'closed' areas (static stomatal patchiness) which is mainly dependent on the size distribution of the stomata within the areoles. Stress factors which affect the leaf water status typically change in their intensity (e.g. during the course of the day) and, therefore, induce dynamic water status changes which can create a heterogeneous and unstable distribution of epidermal turgor. This typically leads to the occurrence of dynamic (i.e. not stable) stomatal patchiness. Persistent strong water stress will lead to a homogeneous stomatal closure all over the leaf. Thus, except for low PPFD conditions or changes in the CO_2 partial pressure of the ambient air, stomatal patchiness is not likely to occur under steady state conditions. At certain stress levels, minor perturbations resulting in rather small heterogeneities of epidermal turgor are sufficient to disturb the stomatal regulation system. This typically leads to the well known phenomenon of stomatal oscillations or waves of stomatal closure moving across the leaf surface and – via the described coordinating mechanism – to pronounced dynamic stomatal patchiness. Since the stomatal regulation mechanism contains components which have the potential to behave chaotically, the latter phenomena can also occur spontaneously.

Acknowledgments. Part of the present work was funded through a Ph.D. scholarship of the Deutsche Forschungsgemeinschaft to J.E. (Graduiertenkolleg: Pflanze im Spannungsfeld zwischen Nährstoffangebot, Klimastreß und Schadstoffbelastung, University of Würzburg, Germany).

REFERENCES

[1] Apel P., Über rhythmisch verlaufende Änderungen in der CO2-Aufnahme von Blättern, Ber. Dtsch. Bot. Ges. 80 (1967) 3–9.

[2] Beyschlag W., Eckstein J., Stomatal patchiness, in: Behnke K., Esser K., Kadereit J.W., Lüttge U., Runge M. (Eds.), Progress in Botany, vol. 60, Springer-Verlag, Berlin-Heidelberg-New York, 1998, pp. 283–298.

[3] Beyschlag W., Pfanz H., A fast method to detect the occurrence of _nonhomogeneous distribution of stomatal aperture in heterobaric plant leaves, Oecologia 82 (1990) 52–55.

[4] Beyschlag W., Phibbs A., Pfanz H., The role of temperature and humidity in controlling the diurnal stomatal behaviour of *Arbutus unedo* L. during the dry season, Biochem. Physiol. Pflanzen 186 (1990) 265–271.

[5] Beyschlag W., Pfanz H., Ryel R.J., Stomatal patchiness in Mediterranean evergreen sclerophylls. Phenomenology and consequences for the interpretation of the midday depression in photosynthesis and transpiration, Planta 187 (1992) 546–553.

[6] Cardon Z.G., Mott K.A., Berry J.A., Dynamics of patchy stomatal movements and their contribution to steady state and oscillating stomatal conductance calculated with gas-exchange techniques, Plant Cell Environ. 17 (1994) 995–1005.

[7] Cowan I.R., As to the mode of action of the guard cells in dry air, in: Schulze E.D., Caldwell M.M. (Eds.), Ecophysiology of Photosynthesis, Ecological Studies, vol. 100, Springer-Verlag, Berlin-Heidelberg-New York, 1994, pp. 205–229.

[8] Daley P.F., Raschke K., Ball J.T., Berry J.A., Topography of

photosynthetic activity of leaves obtained from video images of chlorophyll fluorescence, Plant Physiol. 90 (1989) 1233–1238.
[9] DeMichele D.W., Sharpe P.J.H., An analysis of the mechanics of guard cell motion, J. Theor. Biol. 69 (1973) 77–96.
[10] Downton W.J.S., Loveys B.R., Grant W.J.R., Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid, New Phytol. 108 (1988) 263–266.
[11] Downton W.J.S., Loveys B.R., Grant W.J.R., Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis, New Phytol. 110 (1988) 503–509.
[12] Downton W.J.S., Loveys B.R., Grant W.J.R., Salinity effects on the stomatal behavior of grapevine, New Phytol. 116 (1990) 499–503.
[13] Düring H., Low air humidity causes non-uniform stomatal closure in heterobaric leaves of *Vitis* species, Vitis 31 (1992) 1–7.
[14] Düring H., Loveys B.R., Stomatal patchiness of field-grown sultana leaves: diurnal changes and light effects, Vitis 35 (1996) 7–10.
[15] Dunn D.B., Sharma G.K., Campbell C.C., Stomatal patterns of dicotyledons and monocotyledons, Am. Midl. Nat. 74 (1965) 185–195.
[16] Eckstein J., Heterogene Kohlenstoffassimilation in Blättern höherer Pflanzen als Folge der Variabilität stomatärer Öffnungsweiten, Charakterisierung und Kausalanalyse des Phänomens 'stomatal patchiness', Ph.D. thesis, University of Würzburg, 1997.
[17] Eckstein J., Beyschlag W., Mott K.A., Ryel R.J., Changes in photon flux can induce stomatal patchiness, Plant Cell Environ. 19 (1996) 1066–1075.
[18] Eckstein J., Artsaenko O., Conrad U., Peisker M., Beyschlag W., Abscisic acid is not necessarily required for the induction of patchy stomatal closure, J. Exp. Bot. 49 (1998) 611–616.
[19] Ellenson J.L., Amundson R.G., Delayed light imaging for the early detection of plant stress, Science 215 (1982) 1104–1106.
[20] Epron D., Dreyer E., Photosynthesis of oak leaves under water stress: maintenance of high photochemical efficiency of photosystem II and occurrence of non-uniform CO2 assimilation, Tree Physiol. 13 (1993) 107–117.
[21] Farquhar G.D., Hubick K.T., Terashima I., Condon A.G., Richards R.A., Genetic variation in the relationship between photosynthetic CO2 assimilation rate and stomatal conductance to water loss, Progr. Photosynth. Res. IV5 (1987) 209–212.
[22] Fuchs E.E., Livingston N.J., Hydraulic control of stomatal conductance in Douglas fir (*Pseudotsuga meziesii* (Mirb.) Franco) and alder (*Alnus rubra* (Bong)) seedlings, Plant Cell Environ. 19 (1996) 1091–1098.
[23] Genty B., Meyer S., Quantitative mapping of leaf photosynthesis using chlorophyll fluorescence imaging, Aust. J. Plant Physiol. 22 (1995) 277–284.
[24] Gollan T., Passioura J.B., Munns R., Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves, Aust. J. Plant Physiol. 13 (1986) 459–464.
[25] Gunasekera D., Berkowitz G.A., Heterogeneous stomatal closure in response to leaf water deficits is not a universal phenomenon, Plant Physiol. 98 (1992) 660–665.
[26] Haefner J.W., Buckley T.N., Mott K.A., A spatially explicit model of patchy stomatal responses to humidity, Plant Cell Environ. 20 (1997) 1087–1097.
[27] Jarvis P.G., McNaughton K.G., Stomatal control of transpiration: scaling up from leaf to region, Adv. Ecol. Res. 15 (1986) 1–49.
[28] Jones H.G., Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces, Plant Cell Environ. 22 (1999) 1043–1055.
[29] Laisk A., Oja V., Kull K., Statistical distribution of stomatal apertures of *Vicia faba* and *Hordeum vulgare* and the Spannungsphase of stomatal opening, J. Exp. Bot. 31 (1980) 49–58.
[30] Loreto F., Sharkey T.D., Low humidity can cause uneven photosynthesis in Olive (Olea europaea L.) leaves, Tree Physiol. 6 (1990) 409–415.
[31] Mansfield T.A., Hetherington A.M., Atkinson C.J., Some current aspects of stomatal physiology, Ann. Rev. Plant Physiol. Plant Mol. Biol. 41 (1990) 55–75.
[32] Meidner H., Edwards M., Osmotic and turgor pressures of guard cells, Plant Cell Environ. 19 (1996) 503.
[33] Mott K.A., Effects of patchy stomatal closure on gas-exchange measurements following ABA treatment, Plant Cell Environ. 18 (1995) 1291–1300.
[34] Mott K.A., Cardon Z.G., Berry J.A., Asymmetric patchy stomatal closure for the two surfaces of *Xanthium strumarium* L. leaves at low humidity, Plant Cell Environ. 16 (1993) 25–34.
[35] Mott K.A., Denne F., Powell J., Interactions among stomata in response to perturbations in humidity, Plant Cell Environ. 20 (1997) 1098–1107.
[36] Nonami H., Schulze E.D., Cell water potential, osmotic potential and turgor in the epidermis and mesophyll of transpiring leaves, Planta 177 (1989) 35–46.
[37] Nicolis G., Prigogine I., Exploring Complexity, Freeman, New York, 1989.
[38] Omasa K., Hashimoto Y., Aiga I., A quantitative analysis of the relationships between O_3 absorption and its acute effects on plant leaves using image instrumentation, Environ. Contr. Biol. 19 (1981) 85–92.
[39] Omasa K., Shimazaki K.I., Aiga I., Larcher W., Onoe M., Image analysis of chlorophyll fluorescence transients for diagnosing the photosynthetic system of attached leaves, Plant Physiol. 84 (1987) 748–752.
[40] Passioura J.B., The transport of water from soil to shoot in wheat seedlings, J. Exp. Bot. 31 (1980) 333–345.
[41] Patzke J., Die Heterogenität der Stomaweiten und ihr Einfluß auf die Verteilung des Kohlendioxids und der Photosyntheseaktivität im Blatt: Gaswechselmessungen, Rasterelelektronenmikroskopie, und Chlorophyllfluoreszenz-Bildanalyse, Ph.D. thesis, University of Göttingen, Göttingen, 1990.
[42] Peisker M., Tichá I., Effects of chilling on CO2 gas exchange in two cultivars of *Phaseolus vulgaris* L, J. Plant Physiol. 138 (1991) 12–16.
[43] Pisek A., Knapp H., Ditterstorfer J., Maximale Öffnungsweite und Bau der Stomata mit Angaben über ihre Größe und Zahl, Flora 159 (1970) 459–479.
[44] Pospíšilová J., Šantrucek J, Stomatal patchiness, Biol. Plant 36 (1994) 481–510.
[45] Pospíšilová J., Šantrucek J., Stomatal patchiness: effects on photosynthesis, in: Pessarakli M. (Ed.), Handbook of Photosynthesis, Marcel Dekker Inc., New York, 1997, pp. 427–441.
[46] Raschke K., Leaf hydraulic system: rapid epidermal and stomatal responses to changes in water supply, Science 167 (1970) 189–191.
[47] Raschke K., Stomatal response to pressure changes et inter

ruptions in the water supply of detached leaves of *Zea mays*, Plant Physiol. 45 (1970) 414–423.

[48] Raschke K., Movements of stomata, in: Haupt W., Feinleib M.E. (Eds.), Physiology of Movements, Encyclopedia of Plant Physiology, vol. 7, Springer-Verlag, Berlin-Heidelberg-New York, 1979, pp. 382–441.

[49] Raschke K., Kühl U., Stomatal responses to changes in atmospheric humidity and water supply: experiments with leaf sections of *Zea mays* in CO_2 free air, Planta 87 (1969) 36–48.

[50] Raschke K., Patzke J., Daley P.F., Berry J.A., Spatial and temporal heterogeneities of photosynthesis detected through analysis of chlorophyll-fluorescence images of leaves, in: Baltscheffsky M. (Ed.), Current Research in Photosynthesis, Kluwer Academic Publishers, Boston, 1990, pp. 573–578.

[51] Saliendra N.Z., Sperry J.S., Comstock J.P., Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*, Planta 196 (1995) 357–366.

[52] Scheuermann R., Biehler K., Stuhlfauth T., Fock H.P., Simultaneous gas exchange and fluorescence measurements indicate differences in the response of sunflower, beab and maize to water stress, Photosynth. Res. 27 (1991) 189–197.

[53] Schurr U., Gollan T., Schulze E.D., Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. II. Stomatal sensitivity to abscisic acid imported from the xylem sap, Plant Cell Environ. 15 (1992) 561–567.

[54] Sharkey T.D., Seemann J.R., Mild water stress effects on carbon-reduction-cycle intermediates, ribulose bisphosphate carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves, Plant Physiol. 89 (1989) 1060–1065.

[55] Sharkey T.D., Loreto F., Vassey T.L., Effects of stress on photosynthesis, in: Baltscheffsky M. (Ed.), Current Research in Photosynthesis, Kluwer Academic Publishers, Boston, 1990, pp. 549–556.

[56] Sharpe P.J., Wu H., Spence R.D., Stomatal mechanics, in: Zeiger E., Farquhar G.D., Cowan I. (Eds.), Stomatal Mechanics, Stanford University Press, Stanford, 1987, pp. 91–114.

[57] Siebke K., Weis E., 'Assimilation images' of leaves of *Glechoma hederacea*: Analysis of non-synchroneous stomata related oscillations, Planta 196 (1995) 155–165.

[58] Terashima I., Anatomy of non-uniform leaf photosynthesis, Photosynth. Res. 31 (1992) 195–212.

[59] Terashima I., Wong S.C., Osmond C.B., Farquhar G.D., Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves with having different mesophyll anatomies, Plant Cell Physiol. 29 (1988) 385–394.

[60] Ward D.A., Drake B.G., Osmotic stress temporarily reverses the inhibitions of photosynthesis and stomatal conductance by abscisic acid - evidence that abscisic acid induces a localized closure of stomata in intact, detached leaves, J. Exp. Bot. 199 (1988) 147–155.

[61] Weis E., Berry J.A., Quantum efficiency of photosystem II in relation to energy dependent quenching of chlorophyll fluorescence, Biochim. Biophys. Acta 894 (1987) 198–208.

[62] Wylie R.B., Conduction in the cotyledon leaves, Proc. Iowa Acad. Sci. 53 (1946) 195–202.

[63] Xu D.Q., Terashima K., Crang R.F.E., Chen X.M., Hesketh J.D., Stomatal and nonstomatal acclimation to a CO2 enriched atmosphere, Biotronics 23 (1994) 1–9.