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In situ observation of stomatal movements and gas exchange of *Aegopodium podagraria* L. in the understorey

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Abstract

Observations of stomata *in situ* while simultaneously measuring CO_2 gas exchange and transpiration were made in field experiments with *Aegopodium podagraria* in a highly variable light climate in the understorey of trees. The low background photosynthetic photon flux density (PPFD) caused a slight opening of the stomata and no visible response to sporadic lightflecks. However, if lightflecks were frequent and brighter, slow opening movements were observed. Small apertures were sufficient to allow maximal photosynthetic rates. Therefore, the small apertures observed in low light usually only caused minor stomatal limitations of lightfleck photosynthesis. The response of stomata to step-wise changes in PPFD under different levels of leaf to air vapour pressure difference (Δ_W) was observed under controlled conditions. High Δ_W influenced the stomatal response only slightly by reducing stomatal aperture in low light and causing a slight reduction in the initial capacity to utilize high PPFD levels. Under continuous high PPFD, however, stomata opened to the same degree irrespective of Δ_W. Under high Δ_W, opening and closing responses to PPFD-changes were faster, which enabled a rapid removal of the small stomatal limitations of photosynthesis initially present in high Δ_W after longer periods in low light. It is concluded that *A. podagraria* maintains a super-optimal aperture in low light which leads to a low instantaneous water use efficiency, but allows an efficient utilization of randomly occurring lightflecks.

Key words: Stomata, light environment, CO_2 exchange, transpiration, *Aegopodium podagraria*, stomatal conductance.

Introduction

Changes in stomatal aperture control water vapour loss and CO_2 uptake of leaves according to the availability and demand of these resources. The photosynthetic demand for CO_2 may change rapidly with fluctuations in photosynthetic photon flux density (PPFD), whereas supply and demand for water change relatively slowly. Usually stomatal movements are much slower than the changes in PPFD caused by intermittent cloud cover or moving lightflecks in more or less wind-stirred canopies. Consequently, in the field, stomatal conductance is mostly far from the equilibrium and the transient states of stomatal reactions merit more attention than steady-state reactions (Knapp and Smith, 1990). Stomatal optimization of water use efficiency (WUE) (Cowan, 1977; Farquhar *et al*., 1980) in fluctuating light conditions cannot be assessed from steady-state responses. In fact, it is not the instantaneous optimum, but the long-term integrated CO_2- and water exchange that is important for plant performance.

Plants in the understorey of a forest receive a large portion of photon flux from short lightflecks which interrupt longer periods of low light (Pearcy, 1988). The efficiency with which leaves utilize this light-energy mainly depends on the speed at which full photosynthetic capacity is attained. This photosynthetic induction consists of biochemical processes (Kirschbaum and Pearcy, 1988*a*; Sassenrath-Cole and Pearcy, 1992; Seemann *et al*., 1988), and stomatal opening which removes the CO_2-limitation of photosynthesis (Kirschbaum *et al*., 1988; Tinoco-Ojanguren and Pearcy, 1992, 1993*b*). Whereas the biochemical components of induction occur over the range of seconds to minutes, the removal of stomatal limitation takes several minutes up to more than one hour.

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The function of stomatal responses in photosynthetic induction under understorey conditions has been investigated in a number of studies (Kirschbaum *et al*., 1988; Kirschbaum and Pearcy, 1988*b*; Tinoco-Ojanguren and Pearcy, 1993*b*; Valladares *et al*., 1997). These studies show that delayed stomatal opening may significantly limit carbon gain during short lightflecks, however, it appears that in shade plants specific strategies have evolved which reduce this limitation of photosynthesis while not expending too much water.

In all these investigations of stomatal reactions under ambient conditions, gas exchange techniques were used, which give an integrated response of all stomata on a leaf. These measurements provide information on the effects of stomatal responses on gas exchange. However, for two reasons they convey little information on the responses itself. Firstly, the exact relationship linking conductance to stomatal aperture is usually obscure. Secondly, there is a large variability between reactions of individual stomata which is masked by gas exchange.

Direct observations of stomatal movements, on the other hand, have mostly been made under laboratory conditions on isolated parts of epidermis and on detached leaves and are therefore hardly representative of field conditions. Even experiments on intact potted plants from greenhouses are limited, because this conditioning modifies stomatal reactions in many ways and can lead to artefacts (Assmann, 1992; Kaiser and Kappen, 1997; Talbott *et al*., 1996).

In this study a novel approach was used to overcome these experimental restrictions. The responses of a set of single stomata was investigated together with whole leaf gas exchange *in situ* on leaves attached to essentially undisturbed plants in their natural habitat.

Data are presented here from experiments with *Aegopodium podagraria* L. (Apiaceae), a perennial hypostomatous herb. The field measurements in a shady understorey allow a detailed analysis of stomatal movements in response to a highly variable light environment and their effect on gas exchange.

Materials and methods

The device used for microscopic *in situ* observations and CO_2/H_2O gas exchange measurements on the same leaf that was previously used in growth chamber experiments (Kaiser and Kappen, 1997; Kappen *et al*., 1987; Kappen and Haeger, 1991; Kappen *et al*., 1994) was adapted for field measurements.

The technical details have been described previously (Kaiser and Kappen, 1997). In short, it consists of an inverted video microscope (long-distance objective 40×, Zeiss, FRG) inserted in the bottom of a gas exchange cuvette. A leaf is attached above the microscopic objective in a leaf holder, which is driven by a motorized remote-controlled microscopic stage. A computer program was designed to relocate selected stomata in the focus of the objective repeatedly and to save digitized video-images of the stomatal pore to hard disk. Observation of stomata at low light or in darkness was enabled by the transmitted light of a GaAlAs emitter infrared diode (model OD880F, Optek, US). The wavelength range, which peaks at 880 nm, has only a minor overlap with the absorption spectrum of phytochrome P_{FR} and no overlap with phytochrome P_R and is therefore believed to be practically physiologically inactive. The diode was attached to an arm and by remote control could be moved over the observed leaf region. To avoid shading, it stayed in this position only during the moment of direct inspection.

The images of the stomata (Fig. 1) were taken with the microscope focused to the narrowest part lower down in the pore. The measurements of the pore area were made manually

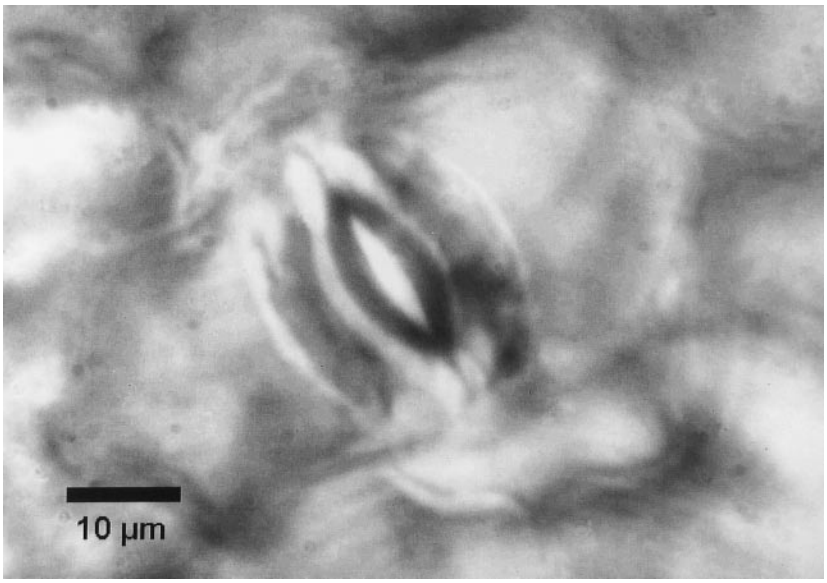

Fig. 1. A stoma of *A. podagraria* as seen in the video microscope for *in situ* observations. The displayed pore has an area of 21 µm^2, a length of 12.3 µm and a width of 2.3 µm. The degree of opening (pore width expressed as percentage of porelength) is 18.7%.

by delineating the pore edge with the cursor. Although time-consuming, this procedure yielded more reliable results than automated measurements based on automatic edge detection. Errors in pore area measurements ranged between ± 0.4 to 0.8 μm^2 ($\pm$ standard deviation), depending on the image quality. This error was nearly independent of the degree of opening, leading to a higher proportional error at low apertures. Apertures of very slightly opened stomata can not be exactly determined. A 'zero' reading therefore can not completely rule out that there is a small stomatal opening left, which nevertheless can significantly contribute to gas exchange (Kerstiens, 1996).

In order to compare the apertures of stomata of different size, a 'degree of opening' was calculated, which expresses pore width in percentage of pore length. This unit of measurement removes the part of the stomatal variation, merely caused by the variation in size. Between 5 and 40 stomata were observed in each experiment, usually randomly sampled from an area of about 2 cm^2 in the middle of the leaf. The entire leaf surface could not be inspected due to the limited flexibility of the petiole which is bent by the movements of the leaf holder.

Gas exchange measurements

The gas exchange equipment (Walz, FRG) is an open flow system which measures transpiration every minute by means of the bypass principle and CO_2 exchange every 2 min with an infrared gas analyser. Humidity and temperature in the cuvette could either be regulated in tracking mode, following the external conditions, or be regulated to a constant value.

Leaf temperature was measured by a 0.2 mm thermocouple attached to the lower leaf surface. The internal fan of the cuvette was set to a moderate speed, producing a boundary layer conductance of about 600 mmol m^{-2} s^{-1} which was measured with a water-saturated filter paper sealed on one side. Gas exchange calculations were performed according to Ball (Ball, 1987). The relatively large cuvette volume (*c.* 4500 cm^3) necessary to enclose the mechanical components for microscopic inspection caused some cuvette lag. The methods described previously (Küppers *et al.*, 1993) were used to estimate the effective cuvette volume and calculate a corrected signal of CO_2 gas exchange.

On some days parallel porometric measurements (model LI-6200, Li-Cor, Lincoln, US) of leaf conductance were made on leaves in the vicinity of the cuvette. In experiments with controlled light, illumination was provided by a fibre optic illuminator (Kaltlicht-Fiberleuchte FL-400, with Spezial Fiberoptik 400-F, Walz, Effeltrich, FRG) or five halogen cold-light lamps (50 W) emitting through a diffuser and supplying a PPFD of up to 700 µmol m^{-2} s^{-1}.

Microclimatic measurements

To measure the PPFD incident on the observed leaf, a small GaAsp Photodiode with a sensitive area of 1.3×1.3 mm (model G2711-01, Hamamatsu, Japan), calibrated against a Li-Cor Quantum Sensor, was mounted inside the cuvette about 12 mm distant from the observed leaf region. Measurements were made every second and the maximum, minimum and average value stored every minute. Relative humidity and air temperature outside the cuvette were measured by a combined humidity/temperature sensor (model HMP 35 AC, Vaisala, FI). Soil water potential was measured at a depth of 25 cm by a tensiometer (Gruler and Trapp, Kiel, FRG). Microclimatic and gas exchange data were recorded by a datalogger (model 21 XL, Campbell, Shepshed, UK) which was supplemented by a multiplexer (model AM 416, Campbell, UK).

Experimental site and plant material

Experiments were made in a dense and uniform stand of non-flowering *A. podagraria* plants in the understorey of a 12 m high canopy of *Carpinus betulus, Tilia cordata, Alnus glutinosa*, and *Populus tremulus* growing in the New Botanical Garden in Kiel (FRG). The site was irrigated during dry periods whenever soil water potential at a depth of 25 cm dropped below -0.5 MPa. The experiments were performed at soil water potentials between -0.18 and -0.5 MPa. Only leaves from non-flowering plants were used, which were enclosed in the gas exchange cuvette for about 1 week, during which several experiments on the same set of stomata were performed. Except during the experimental periods the cuvette conditions tracked the external humidity, temperature and light conditions.

Results

Methodological aspects

In order to ensure that the aperture measurements are representative of the whole stomatal population of the leaf, the occurrence of a patchy distribution of stomatal apertures was checked under conditions which are known to favour this, such as low air humidity or reduced PPFD (Beyschlag and Eckstein, 1998; Eckstein *et al.*, 1996). In five non-adjacent areoles 10 stomata were randomly selected for aperture measurements (Fig. 2a, b). The variance between areoles was normally much smaller than the variance within one areole. In only one out of seven trials was a significant difference between at least two of the five areoles found. These results show that stomatal patchiness is not a frequent phenomenon in *A. podagraria* under the given circumstances.

To check for the possible effects by enclosing the leaf in the cuvette, leaf conductance was measured in parallel on five attached leaves near the cuvette while cuvette conditioning was tracking the external conditions (Fig. 3i). Leaf conductance varied only slightly between leaves and cuvette, and porometric measurements were consistent.

Reactions under natural microclimatic conditions

Daily courses of stomatal reactions and gas exchange under ambient light and simulated external temperature and humidity conditions were recorded on eight days under different weather conditions. Figure 3 shows representative courses of microclimate and leaf responses on two days with fine weather. The light climate was characterized by sustained low light periods (20–80 µmol m^{-2} s^{-1}), which were interrupted by lightflecks with a duration of some minutes and an average PPFD of 300–500 µmol m^{-2} s^{-1}. Leaf flutter caused short-term fluctuations within one lightfleck, with some peak values above 1000 µmol m^{-2} s^{-1}.

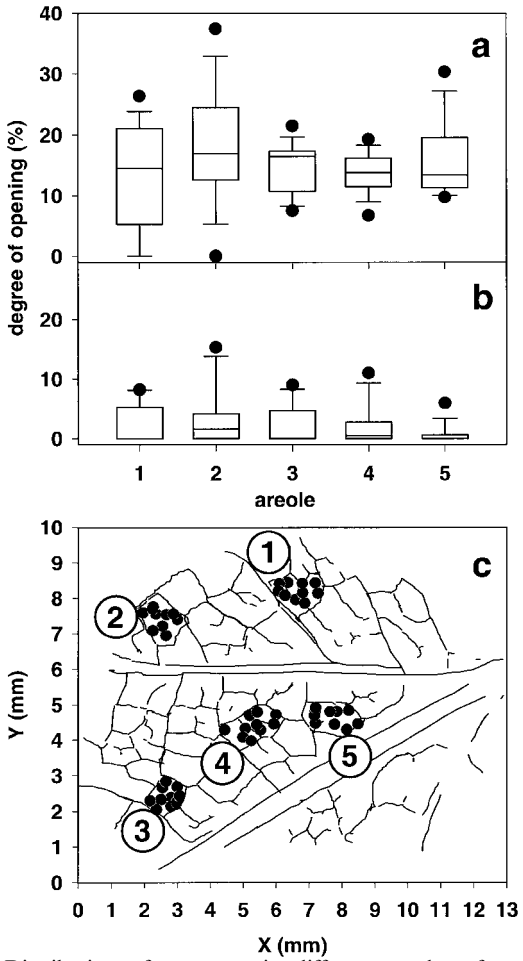

Fig. 2. Distribution of apertures in different areoles of a leaf of *A. podagraria*: (c) the position of the observed stomata in relation to the leaf veins. The boxplots in (a) and (b) depict the distribution of apertures (median, 10th, 25th, 50th, 75th, and 90th percentiles, dots represent outliers) for each observed areole at two times. (a) The apertures after 60 min illumination (PPFD of 500 μmol m^{-2} s^{-1}) and (b) 30 min later after 25 min in low light (18 μmol m^{-2} s^{-1}). The measurements were made at a temperature of 20 °C and a Δ_W of 16 mmol mol^{-1}. No significant differences were found by one-way-ANOVA (a) and the Kruskal–Wallis test (b) at $P = 0.05$.

Stomata reached only small apertures under such conditions. The maximum aperture observed (7 μm^2) was only about 20% of the maximum aperture under continuous saturating light at low Δ_W. Many stomata showed no microscopically visible opening at all. Movements were slow compared with the rapid fluctuations in PPFD. The two days had similar light conditions, but the air humidity was different. During the humid day (Fig. 3a–e), the stomata slowly opened in low light during the morning hours and maintained a rather constant aperture until 14 h although a few lightflecks and a phase of evenly increased PPFD appeared between 11 h and 14 h. On the drier day with steadily increasing Δ_W (Fig. 3f–j), the stomata opened slowly in the humid morning hours, but closed again under continuing low light, when Δ_W was increased. At noon, when Δ_W was about 15 mmol mol^{-1} a period of relatively high PPFD with several lightflecks caused another opening response of a fraction of the observed stomata.

Leaf conductance was generally low, with a maximum at 100 mmol m^{-2} s^{-1}, but it did not drop below 50–60 mmol m^{-2} s^{-1}even on dry and warm days and low PPFD. Even if stomata visually appeared to be closed, the leaf conductance was not at its minimum. Moreover, the decrease of g_L in the afternoon continued when stomata appeared already closed (Fig. 3). This may be because either a small proportion of stomata not seen could still be open, or the small aperture changes below the resolution threshold of the microscope contributed to these changes in conductance.

The dependency of stomatal aperture on Δ_W and PPFD under ambient microclimatic conditions measured in a total of eight diurnal courses is summarized in Fig. 4. In low PPFD stomata could reach large apertures if Δ_W was low, but at high Δ_W only small apertures were observed. However, at high PPFD, stomata opened nearly unaffected by air humidity up to a Δ_W of 15–20 mmol mol^{-1}.

Effect of stomatal aperture on leaf conductance and photosynthetic capacity

To determine the relationship between mean aperture and leaf gas exchange, a leaf was exposed to saturating PPFD (500 μmol m^{-2} s^{-1}) after a period in darkness before noon and pore area of 40 stomata and leaf conductance were measured simultaneously when the stomata were slowly opening (Fig. 5a). The relationship between leaf conductance and maximum photosynthetic rate was analysed in a similar experiment in the late afternoon (Fig. 5b). At this time stomatal opening is very slow and after several minutes exposure to high PPFD photosynthetic rates—at presumably full biochemical induction—could be recorded at a stomatal conductance below 20 mmol m^{-2} s^{-1}. The measurements in the first 12 min were omitted because biochemical induction was assumed to be incomplete. Hyperbolic functions ($y = y_0 + (ax/b + x)$) were used to fit the relationships between aperture and g_L as well as the relationship between g_L and A_{max}. By combining these functions the direct dependence of A_{max} on aperture is described in Fig. 5c.

Figure 5 clearly demonstrates the non-linear relationship between stomatal aperture and gas exchange. Aperture changes have the largest effect on g_L at small apertures: 90% of maximal leaf conductance was reached at a mean pore area by about 10 μm^2. Stomatal movements at higher apertures exert much less control. This is particularly valid for the control on A_{max}: a mean

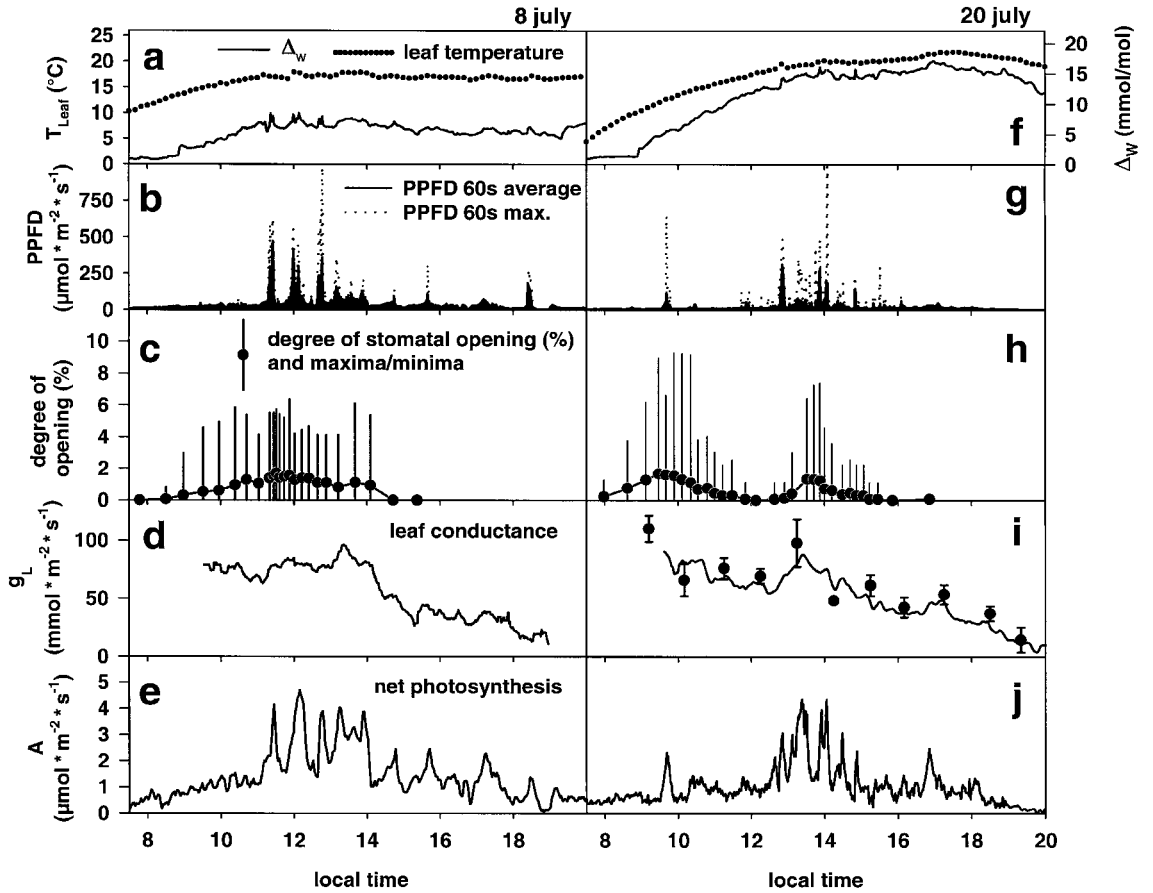

Fig. 3. Diurnal courses of microclimate, stomatal aperture and gas exchange of two leaves of *A. podagraria* in the understorey of trees for 2 d. In (a) and (f) leaf temperature and water vapour difference between leaf and air (Δ_W), in (b) and (g) 60 s average PPFD (black) and 60 s PPFD maxima (dotted) and in (c) and (h) stomatal reactions are depicted. Average apertures of 15 stomata (circles) and the respective maximal and minimal values of the sample (vertical lines) are shown. Courses of leaf conductance are shown in (d) and (i). (d) also shows data of porometric measurements of leaf conductance (circles: average, error bars: standard deviation) on five leaves in the vicinity of the cuvette. Net photosynthesis is graphed in (e) and (j).

aperture of 3 μm^2 already allowed for 90% of photosynthetic capacity.

Stomatal reactions to light periods of different length

The stomatal reaction to light periods (500 $\mu mol\ m^{-2}\ s^{-1}$) lasting for 2, 7, 15, 30, and 60 min each, and alternating with periods of low light (30 $\mu mol\ m^{-2}\ s^{-1}$, 28–60 min), was observed (Fig. 6) to examine the responsiveness to rapid changes in light intensity which are typical for the natural habitat. In low light the stomata stayed slightly open and g_L did not fall below 50–60 $mmol\ m^{-2}\ s^{-1}$. A 2 min lightfleck induced no response and a 7 min one caused only a slight increase in aperture and g_L. To produce a clearly detectable effect, at least 15 min of high PPFD were required. Even after 1 h of high PPFD stomatal movements were still not complete. The closure after a lightfleck was even slower than the opening response. The course of A shows a fast increase during the first minutes of a lightfleck, which can be attributed to the biochemical induction. After this first rapid increase the slower rise of A reflects the decrease of stomatal limitation. Nevertheless, the substantial increase in aperture and g_L during the 30 and 60 min light periods was accompanied by a comparably small increment of A.

The effect of air humidity on stomatal and gas-exchange reactions to a 1 h strong light period

The responses of five stomata to a 1 h light period (500 $\mu mol\ m^{-2}\ s^{-1}$) at different Δ_W are depicted in Fig. 7. At low PPFD (20 $\mu mol\ m^{-2}\ s^{-1}$) before and after the period with high PPFD, stomatal apertures and leaf conductance were lower in drier air than in moist air. Both aperture and conductance show that opening as well as closing reactions occurred faster the drier the air was. Therefore the initial difference between apertures disappeared during the opening reaction and conductance rose to equal values at all air humidities. As a consequence, transpiration was decreased by lowered conductance at high Δ_W only at low light, leading to equal transpiration at $\Delta_W = 15$ and 20 $mmol\ mol^{-1}$. At high PPFD, however, an increase of Δ_W did not reduce transpiration. Photosynthesis in low and high light was

equal in all three Δ_W-treatments, indicating no CO_2 limitation of photosynthesis due to reduction of conductance at low Δ_W. Water use efficiency (WUE), i.e. the relation between CO_2-uptake and water loss, was lower in low than in high PPFD at all humidities. WUE at high light differed with Δ_W-treatments, being lowest at high Δ_W. The rise of photosynthesis after transition to saturating PPFD occurred faster at low Δ_W due to the higher initial conductance. The transient reduction of photosynthesis during the first 5 min at high Δ_W amounted to about 16% (cumulative CO_2 uptake calculated for the first 5 min at $\Delta_W = 20$ mmol mol^{-1} compared to $\Delta_W = 10$ mmol mol^{-1}). This limitation is only transient and is completely lost during the opening response.

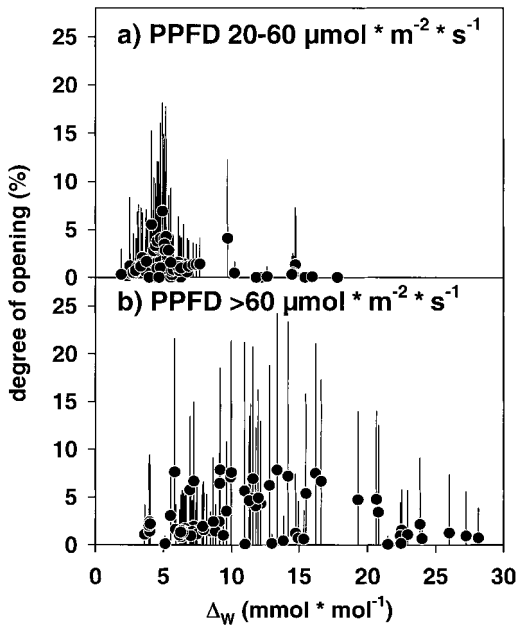

Fig. 4. The dependency of stomatal aperture on Δ_W under low light conditions (a: PPFD = 20–60 µmol m^{-2} s^{-1})and under medium and strong light conditions (b: PPFD > 60 µmol m^{-2} s^{-1}). This figure summarizes data from eight diurnal courses of stomatal aperture of five different leaves under ambient microclimatic conditions. Symbols represent average degrees of opening of 15 stomata. Vertical lines show the range between the minimum and maximum values of the observed sample.

Discussion

The aim of this study was to investigate stomatal responses on undisturbed plants in their natural environment under largely realistic conditions. The cuvette climatization reproduced the natural microclimate with high accuracy. Leaves outside the cuvette had the same leaf conductance as the enclosed leaf during a diurnal course (Fig. 3i), indicating that the enclosure in the cuvette did not seriously affect the stomatal response.

The responses of the observed sample of stomata can be seen as fairly representative because they agreed well with the response of stomatal conductance. Furthermore no indication for stomatal patchiness was found. When considering that large scale-inhomogeneity of apertures may occur (Weyers and Lawson, 1997), it is possible, however, that the sampling of stomata from a limited area in the middle of the leaf led to some bias. Nevertheless, the repeated observation of identical stomata kept this bias constant among experimental treatments and thus does not affect the result of the experiment.

The slow movements and the very low apertures of stomata of *A. podagraria* under natural light conditions may at first sight suggest stomatal dysfunction, as stomatal responses were completely unable to follow the rapid changes in PPFD. Lightflecks needed to be at least as long as 7–15 min to evoke a distinct stomatal response (Fig. 6). To assess the adaptive value of this behaviour, the effect of stomatal aperture on leaf conductance and photosynthetic capacity has to be considered first.

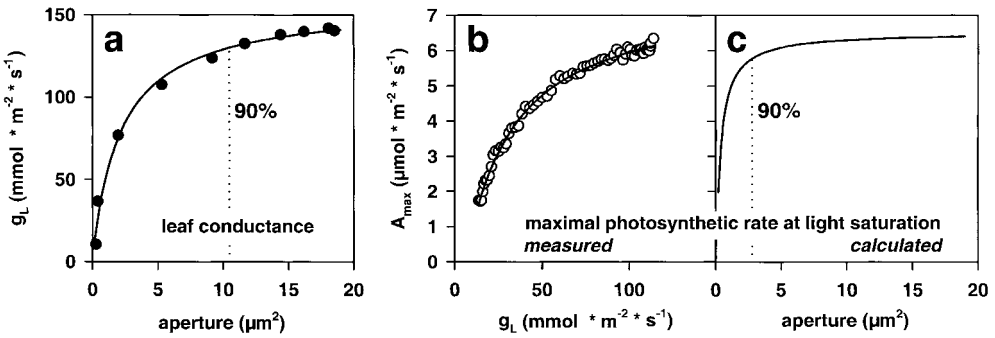

Fig. 5. The dependencies of leaf conductance on mean stomatal aperture (a), of maximal photosynthetic rate at light saturation (A_{max}) on leaf conductance (b) and of A_{max} on stomatal aperture (c). The relationship between leaf conductance and maximum net photosynthesis at saturating light (b) was measured in a separate experiment on the same leaf. Hyperbolic functions were fitted to data in (a) and (b) with $g_L = 3.1821 + (153.952\ Aperture/2.2275 + Aperture)$ and $A = -2.368 + (10.059 g_L/20.878 + g_L)$. The dependency of A_{max} on aperture in (c) was calculated by combining the hyperbolic functions from (a) and (b). Vertical dotted lines indicate apertures which yield 90% of the maximum leaf conductance and photosynthesis, respectively. The cuvette was kept at 16 °C and a Δ_W of 5 mmol mol^{-1} and the leaf received a PPFD of 600 µmol m^{-2} s^{-1}.

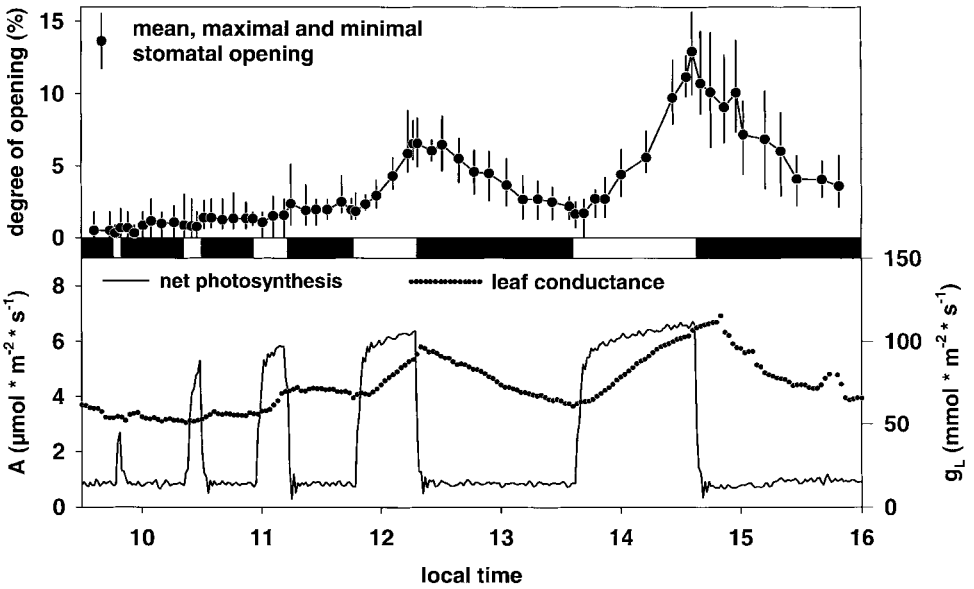

Fig. 6. Stomatal reactions (average and maximum/minimum of the apertures of five stomata) and the response of CO_2/H_2O gas exchange to simulated lightflecks (500 µmol m^{-2} s^{-1}) of different length (2, 7, 15, 30, 60 min). Background light was 30 µmol m^{-2} s^{-1}, cuvette temperature 16 °C and Δ_W 5 mmol mol^{-1}. Light/shade changes are illustrated by the black/white bar between the graphs. In this experiment only stomata were observed which were known to open readily from previous experiments.

Leaf conductance of *A. podagraria* depends on stomatal aperture according to a hyperbolic function with a sharp increase at low apertures and saturation at 50% of maximal aperture. The photosynthetic rate at saturating PPFD (A_{max}) can be also described as a hyperbolic function of g. Therefore, the aperture range which effectively regulates photosynthesis is even narrower than that of g: 90% of maximal photosynthetic capacity are reached at 15% of the maximum observed opening. As a consequence small apertures, induced by the usually low light intensities in the understorey, lead to a leaf conductance which allows for photosynthetic capacity nearly unlimited by CO_2 shortage. If stomatal aperture further increases, photosynthetic rate becomes saturated due to limitation by other factors.

The small range of conductance which limited CO_2 uptake complies with the low photosynthetic capacity of about 7 µmol m^{-2} s^{-1}, which is typical for shade-adapted plants (Larcher, 1994). In fact, when comparing plant species, a positive correlation between photosynthetic capacity and maximal stomatal conductance exists (Wong *et al.*, 1979). In shade-adapted plants this may result in a very narrow aperture range being sufficient for gas exchange regulation. At the lower end of the range leaf conductance varied between 10 and 40 mmol m^{-2} s^{-1} when stomata appeared already closed in the microscope.

These small stomatal movements regulate a large proportion of the naturally occurring range of conductance. Accordingly, fine tuning of stomatal conductance without overshooting responses may require slow opening and closing reactions, as observed in *A. podagraria*.

Stomatal limitation of photosynthesis in lightflecks can roughly be estimated by considering the relationship between aperture, g_L and A_{max} (Fig. 5). Even at high Δ_W leaf conductance in low light was decreased to only about 40 mmol m^{-2} s^{-1}, and still provided two-thirds of maximum photosynthetic capacity. In reality, the effect of stomatal limitation is presumably somewhat smaller, because the incomplete biochemical induction already restricts photosynthesis shortly after transition to high light. Additionally, it is known that post-illuminative CO_2 fixation is increased if the induction state of the leaf is low (Küppers and Schneider, 1993). In these experiments the decreased leaf conductance in low light at Δ_W of 20 mmol mol^{-1} (Fig. 7) led to a moderate lowering of carbon gain by about 15% during the first 5 min in saturating PPFD. A more detailed analysis of the different components of photosynthetic induction (Küppers and Schneider, 1993; Tinoco-Ojanguren and Pearcy, 1993*b*) was not possible with this large-volume system.

The same strategy of maintaining high photosynthetic induction has also been observed in other shade plants. Ögren and Sundin found that *Paris quadrifolia* and *Maianthemum bifolium* compared to species adapted to high light had faster photosynthetic induction because of high initial leaf conductances (Ögren and Sundin, 1996). *Piper hispidum*, a shrub of the humid tropics, showed nearly no reactions to light changes, but responded strongly to changes in high air humidity (Mooney *et al.*, 1983). In consequence, this led to a high conductance in shade periods, whenever air humidity was sufficiently high. Valladares *et al.* observed that high initial leaf

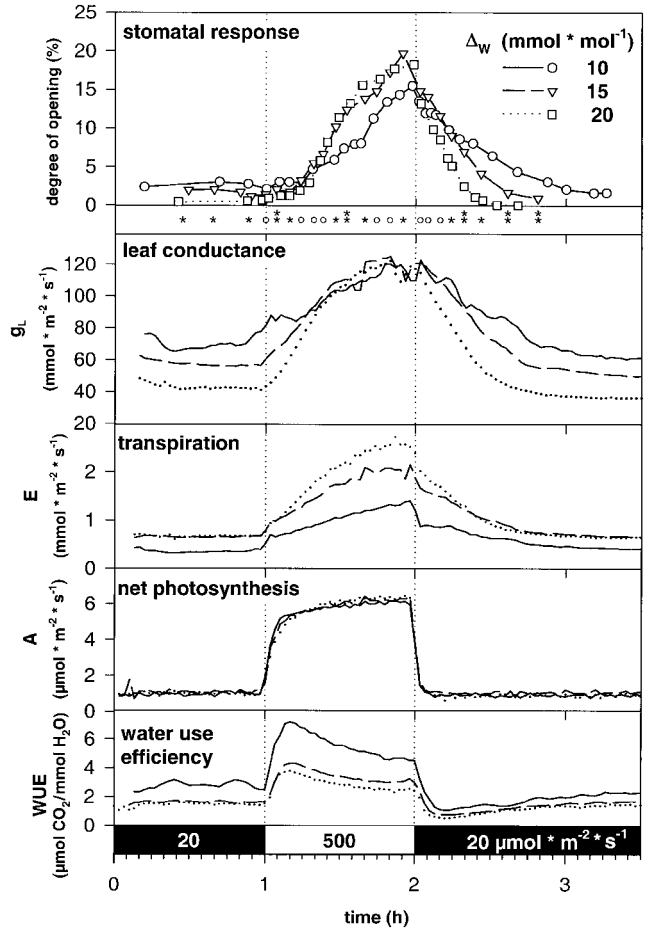

Fig. 7. Comparison of stomatal responses and courses of leaf conductance, transpiration, net photosynthetic rate, and water-use efficiency to 1 h saturating PPFD at different leaf to air water vapour differences. Δ_W was 10, 15 and 20 mmol mol^{-1}, respectively, measured when the leaf was illuminated. In low light Δ_W was 2–5 mmol mol^{-1} lower. PPFD changed from 20 to 500 and back to 20 µmol m^{-2} s^{-1}. Air temperature was 20 °C. The three experiments were performed in the morning on consecutive days. Always the same five stomata were observed and averages computed. Asterisks indicate significant aperture differences as tested by Friedmans test, or if normal distribution could be assumed, by two way (repeated measures) ANOVA ($^{*}P<0.05$, $^{**}P<0.01$). Circles indicate no significant difference between the treatments.

conductance was the most important determining factor for fast photosynthetic induction in a number of neotropical rainforest shrubs (Valladares *et al.*, 1997).

A quite different stomatal behaviour which raises the probability that a lightfleck meets a leaf with opened stomata seems to be typical for shade plants from the tropical understorey. A single lightfleck induces a rapid and hysteretic opening response with an opening movement peaking up to 20 min after the end of a lightfleck, followed by a slow closure (Kirschbaum *et al.*, 1988; Tinoco-Ojanguren and Pearcy, 1992). While photosynthesis during single lightflecks usually does not take benefit from this hysteretic stomatal opening, subsequent lightflecks can be utilized more efficiently at the cost of an increased transpiration in low light periods. This strategy is an example that temporal deviations from an optimal WUE may be favourable under non-steady conditions.

The type of interaction between PPFD and Δ_W further supports the view that *A. podagraria* maximizes carbon gain at the cost of water loss. High Δ_W caused no reduction of aperture in continuous high light, which led to increased water losses (Figs 4, 7). Only in low light high Δ_W caused a slight stomatal closure, which however did not inhibit photosynthesis in low light at all and imposed only slight limitations on the potential to utilize lightflecks. Removal of these stomatal limitations of photosynthesis was supported by faster opening movements under high Δ_W. Conversely, closure was also speeded up in dry air. This effect has been observed in different species (Assmann and Grantz, 1990; Barradas *et al.*, 1994; Tinoco-Ojanguren and Pearcy, 1993*a*) and seems to be brought about by water relations in the epidermis (Kappen *et al.*, 1987; Shackel and Brinckmann, 1984). Although apparently advantageous, this property is therefore better described as a mechanistic effect rather than as an evolutionary adaptation.

Summing up, the strategy of *A. podagraria* under fluctuating light seems to be to maintain sufficient conductance even at the expense of transpired water during periods of low light. Stomatal regulation in shade periods therefore does not lead to an instantaneous optimal WUE, but improves the capacity to utilize sunflecks efficiently in a 'pre-emptive' manner.

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