Photosynthetic Induction and Leaf Carbon Gain in the Tropical Understorey Epiphyte, *Aspasia principissa*

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Gas exchange of the understorey epiphyte *Aspasia principissa* was studied in fluctuating light conditions both in the laboratory and in the field, testing the hypothesis that vascular epiphytes differ from most terrestrial understorey plants in showing a higher priority for water conservation. Consequently, a slow response of stomatal conductance to sudden increases in incident photon flux density (PFD) was expected, as was a fast loss of induction after such a light fleck. Results were only partly consistent with these expectations. Full induction of photosynthesis was indeed very slow and was not reached before, respectively, 40 and 60 min of saturating PFD in the field and the laboratory. In contrast, kinetics of induction loss were comparable with those of most terrestrial species studied to date. The overall impact of light flecks on *in situ* carbon gain again fulfilled expectations, being rather limited: the observed carbon gain was only approx. 66 % of the potential carbon gain estimated from a square-wave response model. It is concluded that in the drought-prone epiphytic habitat of a moist lowland forest, water conservation takes priority over carbon gain, which severely limits the use of light flecks for CO_2 fixation in vascular epiphytes.   © 2003 Annals of Botany Company

INTRODUCTION

Plants in the understorey of many tropical and temperate forests receive very low levels of diffuse light, punctuated from time to time by bright light flecks (Chazdon and Fetcher, 1984). The capacity of shade-adapted plants to exploit these brief periods of high photon flux density (PFD) for photosynthetic carbon gain has received considerable attention in the last two decades (e.g. Chazdon and Pearcy, 1986*a*, *b*; Pearcy, 1990; Ögren and Sundin, 1996; Pearcy *et al*., 1997; Valladares *et al*., 1997; Allen and Pearcy, 2000). These studies have shown that understorey plants typically exhibit photosynthetic adaptation and acclimation that maximize carbon gain, frequently at the expense of increased transpiration. Rather high stomatal conductance (g_w) during periods of low light leads to increased water loss, but improves the capacity to utilize sun flecks (Pfitsch and Pearcy, 1989*b*; Kaiser and Kappen, 2000).

Many vascular epiphytes share the understorey environment with terrestrial herbs (e.g. Chazdon and Pearcy, 1986*b*; Seeman *et al*., 1988), shrubs (Tinoco-Ojanguren and Pearcy, 1993; Allen and Pearcy, 2000) and tree seedlings (Kursar and Coley, 1993). Whilst some of these have been investigated with respect to light fleck utilization, no information is available on the response of epiphyte leaves to fluctuating light conditions in the understorey. Even under moist tropical conditions the members of this plant

group, especially the so-called 'bark epiphytes' that completely lack canopy soil, are subject to frequent water shortage (Benzing, 1990). In view of the overriding importance of water scarcity in the epiphytic habitat (Zotz and Hietz, 2001), we expected that epiphytes such as the understorey orchid *Aspasia principissa* would respond differently to fluctuating light than most terrestrial species. Thus, we predicted that stomata in this species should severely restrict the utilization of brief light flecks by causing longer induction times and faster loss of induction than usually observed in co-occurring understorey shrubs and tree saplings. This question was addressed in both the field and the laboratory. We also predicted a rather limited contribution of carbon gain during light flecks to the overall CO_2 budget. This latter question was addressed by direct documentation of diel net CO_2 exchange under naturally fluctuating environmental conditions in the field.

MATERIALS AND METHODS

Study organisms

Aspasia principissa Reichb. f. (Orchidaceae) occurs in central and eastern Panama, preferring the wetter forests of the region (Croat, 1978). The species is mostly found in the understorey, growing on trunks of various tree species from near ground level up to approx. 15 m. Given that the photosynthetic capacity of this and other epiphyte species varies with plant size (Schmidt *et al*., 2001), mature leaves of larger specimens were used consistently (longest

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pseudobulb, 8–11 cm) both in field and laboratory measurements.

Laboratory studies

Four plants that had been cultivated in a glasshouse at the University of Würzburg, Germany for more than a year were used for the laboratory studies. Glasshouse conditions were approximately as follows: 26/23 °C day/night, 60 % relative humidity (RH), 7 mol m^{-2} d^{-1} PFD. All gas exchange measurements were made using a 'mini-cuvette system' (Walz, Effeltrich, Germany), in which an area of leaf approx. 14–16 cm^2 was enclosed. This system has a rather high internal gas volume (cuvette volume 260 ml). Moreover, due to the low assimilation rates of *Aspasia principissa* it was not possible to use flow rates >0·5 l min^{-1}. Under these conditions the delay between cuvette and infrared gas analyser was approx. 35 s. This period was determined by introducing a small volume of high CO_2 (approx. 2 %) into the cuvette and noting the delay in response of the infrared gas analyser. All data were corrected for this lag time. Since this study did not focus on short-term dynamics of induction kinetics on a scale of seconds, correction was not made for the underestimation of peaks due to mixing in the cuvette as suggested by Pearcy *et al.* (1985).

After investigating the light response of net CO_2 exchange to determine saturating PFD, the following experiments were conducted. For induction response experiments leaves were enclosed in the cuvette and illuminated with approx. 30 µmol m^{-2} s^{-1} PFD, measured at the height of the leaf, for at least 30 min. The temperature in the cuvette was 25 °C, and RH 80–90 %. After obtaining a stable steady-state reading under low light conditions (NP_L), leaves were exposed to saturating PFD (approx. 210 µmol m^{-2} s^{-1}). During the induction period the difference between the CO_2 concentration of air leaving the cuvette and that of the reference stream (ΔCO_2) was recorded continuously using a chart recorder, while full data sets were collected at 2-min intervals until a stable maximum assimilation rate (NP_{max}) was reached. Besides NP_{max}, the following parameters were determined: time to reach 50, 90 and 100 % of full induction, and the initial ($g_{initial}$) and maximum stomatal conductance (g_{max}). Measurements were conducted throughout the day. In contrast to the findings of Allen and Pearcy (2000), the time to reach full induction did not depend on the time of day (*t*-test for independent samples before and after noon, d.f. = 5, P = 0·96). During an induction experiment a given rate of net CO_2 exchange ($NP*$) was expressed as relative induction state following Chazdon and Pearcy (1986*a*):

$$I = (P* - P_L)/(P_{max} - P_L) \times 100 \%$$

where *I* is relative induction state, $P*$ is $NP*$, P_L is NP_L and P_{max} is NP_{max}. Leaves used to determine induction loss rates were pre-treated with saturating light (approx. 250 µmol m^{-2} s^{-1} PFD) for at least 60 min. The leaf was then enclosed in the cuvette under saturating light until a steady-state NP_{max} was attained. Leaves were subsequently shaded (PFD

approx. 30 µmol m^{-2} s^{-1}) for periods of 3, 5, 10, 15, 30 and 60 min. After the shade treatment, leaves were again exposed to saturating PFD. The net rate of CO_2 uptake after 60 s was recorded and used to calculate the induction state (see above).

Use of an oxygen electrode allows photosynthetic activity to be measured in the absence of stomatal limitation, which enabled us to study the role of stomata in limiting net CO_2 uptake during induction (compare Kursar and Coley, 1993). Photosynthetic O_2 exchange was measured using a Hansatech LD2 Leaf Disc Electrode (Hansatech Ltd, Kings Lynn, UK). The electrode chamber, which was kept at 25 °C using a circulating water bath, was charged with 5 % CO_2 saturated with water vapour prior to measurements. Each measurement consisted of a calibration, a period of 15 min in which leaf discs were supplied with 30 µmol m^{-2} s^{-1} incident PFD, and (after recharging the cuvette with 5 % CO_2) a step change in PFD to 300 µmol m^{-2} s^{-1} until full induction was observed.

Field work

Field work was conducted on Barro Colorado Island (BCI; 9°10′N, 79°51′W), Republic of Panama. The vegetation of this biological reserve, which is classified as 'tropical moist forest' (Holdridge *et al.*, 1971), receives approx. 2600 mm precipitation annually, with a pronounced dry season from late December to April. Croat (1978) and Leigh *et al.* (1982) give detailed descriptions of vegetation, climate and ecology.

In March 2000 measurements of CO_2 and H_2O exchange were performed on a total of six plants situated in the understorey approx. 30 m away from the laboratory clearing. One plant was growing naturally on the trunk of a large *Anacardium excelsum* tree at a height of approx. 2 m. The other plants had been fixed to two large branch pieces (approx. 10 cm diameter) about 6 months earlier and had been left in the understorey close to the same tree. All plants were irrigated vigorously by spraying the roots with rain water several times a day for a week before the measurements; irrigation continued during the measurements. As described in detail by Zotz and Winter (1994), leaf gas exchange was studied using a CO_2/H_2O-porometer (CQP 130; Walz) operating in a continuous open-flow mode. CO_2 and water vapour exchange were measured using an infrared gas analyser operating in differential mode (Binos 100P; Rosemount, Hanau, Germany). The same instrument allowed the determination of ambient CO_2 partial pressure. External temperature and relative humidity were tracked inside the leaf cuvette (cuvette volume 188 ml). Small-scale variation in photosynthetic photon flux density (PFD) can be substantial. To minimize such heterogeneity, a leaf area of only approx. 8 cm^2 was enclosed in the cuvette, and PFD was measured immediately adjacent to the studied leaf (distance approx. 1·5 cm). Each study leaf was enclosed for 24-h periods from dusk to dusk. Both PFD and ΔCO_2 were recorded continuously using a chart recorder, but full data sets were only registered every 90 s (daytime) and 20 min (night-time), respectively. Care was taken to maintain leaves in their natural position. The delay between cuvette

and infrared gas analyser was approx. 20 s. After each day course, photosynthetic induction was studied using the same leaf (cuvette temperature 25–27 °C; RH 70–80 %). Shortly after dawn the following morning the leaf was artificially illuminated with approx. 30 μmol m^{-2} s^{-1} PFD. After 30 min, PFD was rapidly increased to 210–220 μmol m^{-2} s^{-1}, i.e. saturating light. After full induction was achieved, the response of leaf CO_2 exchange to different PFDs was studied, starting with 0 μmol m^{-2} s^{-1} to approx. 1000 μmol m^{-2} s^{-1} in ten steps. Experiments were always finished by noon. Gas exchange parameters were calculated following Von Caemmerer and Farquhar (1981).

In addition, changes in induction kinetics under drought stress were studied by determining photosynthetic induction as described above for 7 d without irrigation using three leaves of three different plants. The induction response was determined as described above.

The measurement of the response of net photosynthesis rate (*NP*) to PFD and the application of a modified Smith-function (Smith, 1937; Tenhunen *et al.*, 1976) allowed the development of a simple empirical model of steady-state CO_2 gas exchange. In this model we assumed a square-wave response of *NP* to PFD, i.e. *NP* changed immediately with a change in light conditions. Similar models have been used, for example, by Pfitsch and Pearcy (1989*a*). This model was not intended to simulate real *in situ* CO_2 exchange (compare e.g. Stegemann *et al.*, 1999), but to provide a null model of carbon gain of well-watered plants assuming no need for induction. No attempt was made to include the photosynthetic response to temperature because *in situ* temperatures were rather close to those used during the determination of the light response curves, and preliminary measurements indicated little change in *NP* over the temperature range observed in the field. Following other authors, a light fleck was defined as a period in which PFD exceeded 50 μmol m^{-2} s^{-1} (e.g. Chazdon and Fetcher, 1984; Pfitsch and Pearcy, 1989*a*).

Data analysis

Statistical analyses were performed using STATISTICA software (STATISTICA 5.1; StatSoft Inc., Tulsa, OK, USA).

RESULTS

Steady-state CO₂ exchange

The steady-state response of net CO_2 exchange to PFD was typical of that of a shade plant (Fig. 1): the average light compensation point of six leaves of six plants growing in the understorey on BCI was 6 $\pm$ 2 μmol m^{-2} s^{-1} PFD (mean $\pm$ s.e.), saturation was reached at 155 $\pm$ 44 μmol m^{-2} s^{-1} PFD, and the apparent quantum yield was 0·040 $\pm$ 0·004 mol CO_2 mol^{-1} photons. Even for a shade plant NP_{max} was rather low at 2·3 $\pm$ 0·6 μmol CO_2 m^{-2} s^{-1}.

Time course of photosynthetic induction and induction loss

The time to reach full induction was slow in this epiphyte (Table 1). On average, it took more than 60 min for
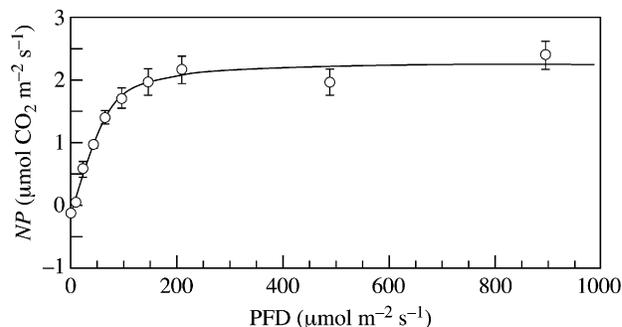

FIG. 1. Photosynthetic light response curves of fully mature leaves of *Aspasia principissa*. Data are means $\pm$ s.e. (*n* = 6). The regression line was determined using the modified Smith-function (r^2 = 0·99, compare Tenhunen *et al.*, 1976).

maximum rates of net CO_2 uptake (P_{max}) to be achieved in glasshouse-grown plants; 90 % of NP_{max} was reached after approx. 40 min. Similar experiments on plants growing naturally in the field yielded a reaction approx. 40 % faster (Table 1). Laboratory- and field-grown plants did not differ in the maximum rates of net CO_2 uptake nor in stomatal conductance (Table 1), which were both quite low. Furthermore, there was no difference in the induction rates when alternating periods of saturating and low light (5 min high, 3 min low) were given rather than continuous light (data not shown). Figure 2 gives a representative example of a measurement in the field. Following an increase in PFD, *NP* increased curvilinearly, reaching a maximum after approx. 35 min. In contrast, stomatal conductance increased almost linearly for the entire 50-min period. The internal CO_2 concentration (c_i) declined from an initial value of approx. 320 μl l^{-1} to 280 μl l^{-1}, to rise slowly to approx. 300 μl l^{-1}. Although these data are suggestive of a non-stomatal component limiting photosynthesis during the first part of the induction process, calculations of c_i at low g_w are subject to considerable doubt. Therefore, the possible role of stomatal limitation in the induction process was explored with measurements of leaf discs in the oxygen electrode. An increase in PFD from 27 to 300 μmol m^{-2} s^{-1} led to an almost instantaneous increase in oxygen evolution. The delay in four replicate experiments was never more than 60 s.

The loss of induction in low light followed a typical pattern resembling a negative exponential function (Fig. 3). The induction state (IS_{60}) was indistinguishable from background (= low light) levels after approx. 60 min.

Diel courses of in situ *carbon gain*

The light climate at the understorey growing site of *Aspasia principissa* on BCI was characterized by low PFD (20–30 μmol m^{-2} s^{-1} for most of the day) interrupted by light flecks of variable duration and intensity. Continuous recording of PFD using a chart recorder revealed much more short-term variation in PFD than is shown in Fig. 4. For example, during the cluster of light flecks between 1000 and 1035 h, maximum PFD values reached 610 μmol

T A B L E 1. *Induction times for glasshouse- and field-grown plants of* Aspasia principissa, *along with maximum rates of net CO_2 uptake and initial and maximum stomatal conductances*

Parameter	Glasshouse-grown plants	Field-grown plants	*P*-value
NP_{max} (μmol m^{-2} s^{-1})	2·8 ± 0·2	2·4 ± 0·2	0·17
$g_{initial}$ (mmol m^{-2} s^{-1})	9·1 ± 2·2	8·2 ± 1·2	0·64
g_{max} (mmol m^{-2} s^{-1})	24·2 ± 6·9	26·5 ± 2·7	0·69
Time to reach 50 % induction (min)	19·8 ± 1·7	8·8 ± 1·0	<0·001
Time to reach 90 % induction (min)	43·7 ± 3·9	25·2 ± 1·9	<0·001
Time to reach full induction (min)	65·2 ± 5·7	39·3 ± 4·2	<0·001

Data are means ± s.e. The significance of differences were tested with *t*-tests for independent samples, d.f. = 14.
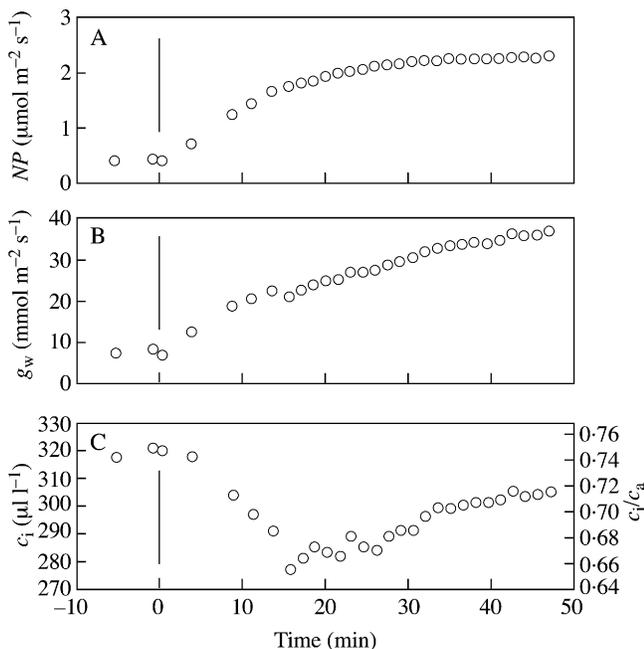

FIG. 2. Representative time course of net CO_2 exchange (A), stomatal conductance (B), and intercellular CO_2 pressure and the ratio of internal to ambient CO_2 (c_i/c_a) (C) following an increase in PFD from approx. 30 to 210 μmol m^{-2} s^{-1} (time = 0 min). Leaf temperature was approx. 27 °C, ambient CO_2 was 421–429 μl l^{-1} and leaf-to-air vapour pressure deficit was 8·6–10·9 mPa Pa^{-1}. Vertical lines indicate the start of saturating PFD.
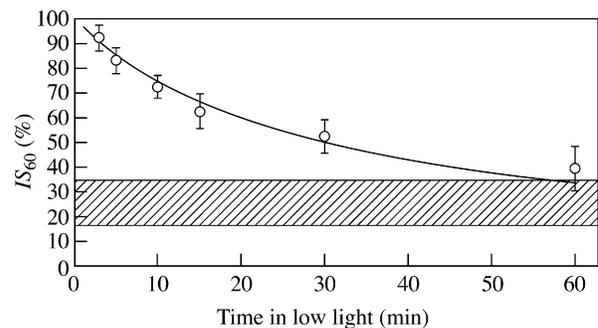

FIG. 3. The loss of induction state (IS_{60}) as a function of time in low light. Leaves were fully induced prior to shading. Data are means ± s.e. (n = seven leaves from four plants). For comparison, the range of IS_{60} under continuous shade conditions (30 μmol m^{-2} s^{-1} PFD) is also given (hatched area). The non-linear regression is $IS_{60} = 1 - (time/(time + 29·8))$ ($r^2 = 0·99$), indicating that 50 % loss of induction is reached after approx. 30 min.

m^{-2} s^{-1} for short periods, but light was also at background levels three times for 1–2 min in the same period. Although Fig. 4 does not show fully the short-term temporal variation in PFD, it adequately represents the three clusters of light flecks during this day: there were only very few additional light flecks of brief duration (<1 min) and of rather low intensity (<100 μmol m^{-2} s^{-1}). Other environmental variables ranged as follows: RH, 65–90 %; air temperature, 22–29 °C; ambient CO_2, 400–429 μl l^{-1}. Although plants were watered regularly during the entire measurement period, stomatal response to light flecks was slow, especially in the afternoon. Maximal stomatal conductance on 10 Mar. 2000 was 26 mmol m^{-2} s^{-1}. The resulting ratio of internal to external CO_2 concentration (c_i/c_a) was around 0·8 for most of the day, but decreased to <0·6 during a long-lasting light

fleck in the early afternoon. In Fig. 4C the measured diel pattern of net photosynthesis is compared with an assumed steady-state response of NP to changing PFD, using data obtained subsequently from a light response curve. The maximum rate of net CO_2 uptake (1·0 μmol m^{-2} s^{-1}) during the 24-h period was considerably lower than the NP of the same leaf after proper induction the following day (2·4 μmol m^{-2} s^{-1}).

Similar diel courses were obtained for mature leaves of four additional plants. Table 2 summarizes integrated daily PFD, diurnal (NP_{day}) and diel leaf carbon budgets ($NP_{24 h}$). Integrated PFD varied from 1·2–2·6 mol m^{-2} d^{-1}, with light flecks accounting for 30–71 % of the total. NP_{day} was inversely related to PFD, but this relationship was only marginally significant ($r = -0·85$, $P = 0·07$). After subtracting light fleck photon flux density, there was no correlation between PFD and NP_{day} ($r = 0·36$, $P = 0·55$). Table 2 also provides estimates of diurnal carbon gain assuming a steady-state response of NP. On average, this was about 50 % more than the measured CO_2 gain in the light (Table 2). The discrepancy between 'expected' steady-state and 'observed' carbon gain was much more pronounced in the afternoon than in the morning: while NP was only slightly below steady-state integrals before 1200 h, it was only half the value in the afternoon (Table 2). This was not a consequence of stronger or longer light flecks in the

afternoon (Fig. 4) because on other days the leaves received more PFD in the morning (e.g. 29 Feb. 2000 morning = $1{\cdot}25$ mol m^{-2} s^{-1} *vs.* afternoon = $0{\cdot}84$ mol m^{-2} s^{-1}).

Drought caused a gradual decline in *NP* and g_w (Fig. 5). Initially, this decline was quite subtle in *NP*, while there was no change at all in g_{max} during the first 3 d without irrigation. g_{max} then decreased linearly until stomata remained almost completely closed on day 7. In contrast to these changes in g_w, the decrease in NP was less pronounced and was delayed. Remarkably, the time to reach full induction was almost constant during the first 6 d of the experiment, ranging from 43 to 53 min. Only on day 7, when stomata hardly reacted to increased PFD, was this time reduced to about 50 %. The induction curve showed the same (hyperbolic) shape throughout the experiment.

DISCUSSION

Vascular epiphytes, in particular bark epiphytes such as *Aspasia principissa*, generally suffer much more frequently from a shortage of water supply than most co-occurring terrestrial species (Zotz and Hietz, 2001). Since an efficient use of light flecks for carbon gain coincides with increased transpirational loss (Kaiser and Kappen, 2000), we anticipated that *A. principissa* would show a slow induction of net
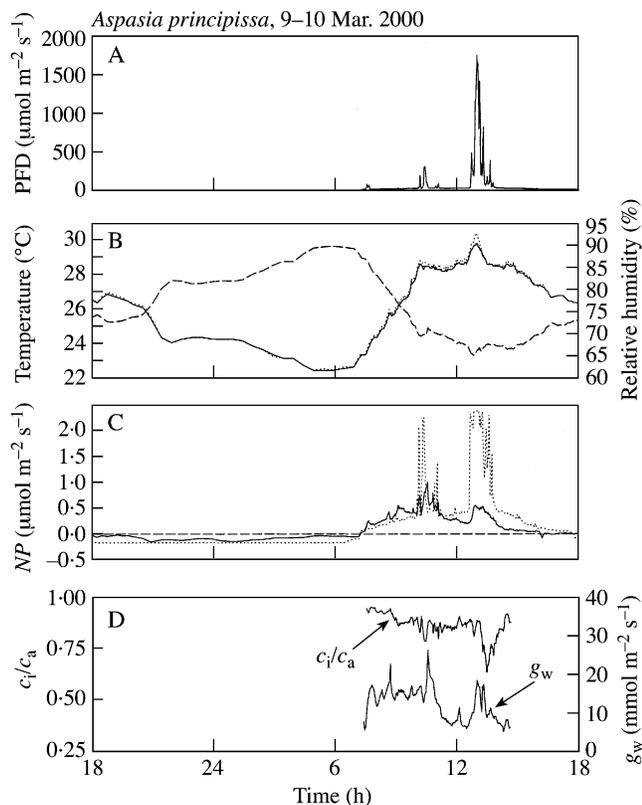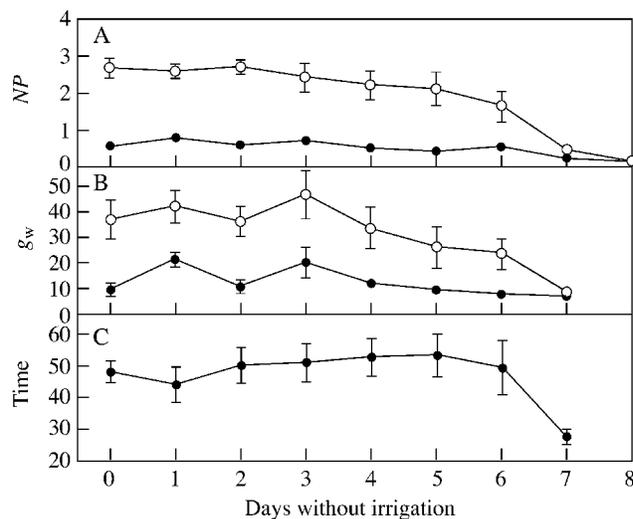

FIG. 4. Diel course of gas exchange parameters and microclimatic conditions of *Aspasia principissa* in the understorey of Barro Colorado Island. A, Incident PFD (μmol m^{-2} s^{-1}); B, leaf (dotted line) and air (continuous line) temperature, and air relative humidity (hatched line); C, net rate of CO$_2$ uptake (μmol m^{-2} s^{-1}) and modelled rates of net CO$_2$ exchange (dotted line, see text); D, ratio of internal to external CO$_2$ concentrations (c_i/c_a) and stomatal conductance to water vapour (g_w, mmol m^{-2} s^{-1}).

FIG. 5. Changes in parameters related to photosynthetic induction during the course of a drought experiment. Data are means ± s.e. (*n* = 3). A, Net rates of CO$_2$ uptake (*NP*, μmol m^{-2} s^{-1}) in low (closed symbols) and saturating PFD (open symbols); B, initial (closed symbols) and maximum (open symbols) stomatal conductance to water vapour (g_w, mmol m^{-2} s^{-1}); C, time to reach full induction (in minutes).

Table 2. *Integrated gas exchange parameters for mature leaves of* Aspasia principissa

Date	PFD	PFD*	$NP_{24\,h}$	NP_{day}	$NP_{steady\text{-}state}$	$NP_{measured}/NP_{steady\ state}$ (%)		
						Entire light period	Dawn–noon	Noon–dusk
27 February	1·8	1·09	10·5	12·7	16·4	77	103	54
29 February	2·1	0·72	1·7	7·7	14·3	54	74	41
2 March	1·2	0·88	13·9	17·1	27·4	62	67	57
9 March	1·6	0·78	14·5	16·8	19·4	86	99	63
10 March	2·6	0·75	5·7	9·5	18·6	51	93	24
Means ± s.e.	1·9 ± 0·2	0·86 ± 0·07	9·3 ± 2·5	12·8 ± 1·9	19·2 ± 2·2	66 ± 7	87 ± 7	48 ± 7

PFD, Diurnal PFD (mol m^{-2} d^{-1}); PFD*, PFD excluding all light flecks >50 μmol m^{-2} s^{-1} (mol m^{-2} d^{-1}); $NP_{24\,h}$, diel carbon budget (mmol m^{-2} d^{-1}); NP_{day}, diurnal carbon gain (mmol m^{-2} 12 h^{-1}); $NP_{steady\text{-}state}$, estimated diurnal carbon gain assuming steady-state response to changes in PFD (mmol m^{-2} d^{-1}).

CO_2 uptake following a sudden increase in PFD, and also a rather rapid loss of induction after such a light fleck. These expectations were borne out in part. Induction was indeed slow: most understorey shrubs and herbs reach full induction much faster than this epiphytic orchid (often in less than 20 min; Pearcy and Calkin, 1983; Chazdon and Pearcy, 1986*a*; Pearcy, 1988; Pfitsch and Pearcy, 1989*b*; Kursar and Coley, 1993; Ögren and Sundin, 1996; Valladares *et al*., 1997). There are, however, a few reports of similarly slow induction times in terrestrial understorey species (e.g. approx. 35 min for *Rheedia edulis*; Kursar and Coley, 1993), while induction kinetics of plants from more exposed habitats are generally comparable with those of *A. principissa* or are even slower (Ögren and Sundin, 1996). Our finding of a significant difference between plants growing in the glasshouse and those in their natural environment (Table 1) indicates the necessity for caution when interpreting data from laboratory studies, in particular because similar observations have been made before. Clearly, glasshouse conditions or cultivation in pots may alter stomatal reactions to a number of environmental variables (Assmann, 1992; Talbott *et al*., 1996). For example, Kursar and Coley (1993) reported that the response of forest plants is two to seven times quicker than that of potted conspecifics. The opposite trend was found by Ögren and Sundin (1996), who attributed this to the harsher conditions in the field. This interpretation can hardly be used in the case of *Aspasia*: like Kursar and Coley (1993), we are unable to offer a convincing explanation for our finding.

The almost immediate rise in O_2 evolution upon an increase in PFD suggests that the slow induction in *Aspasia* is mostly a consequence of stomatal limitations, while biochemical aspects (activation of ribulose-1,5-bisphosphate carboxylase-oxygenase and regeneration of ribulose-1,5-bisphosphate; Pearcy, 1990) play a minor role. The range of stomatal movements in *Aspasia* is much lower than that in most terrestrial understorey herbs, shrubs and tree saplings (Chazdon and Pearcy, 1986*a*; Pfitsch and Pearcy, 1989*b*; Valladares *et al*., 1997; Allen and Pearcy, 2000), the difference between g_w in the shade and at saturating PFD being less than 20 mmol m^{-2} s^{-1} (Table 1). Kaiser and Kappen (2000) suggested that under such conditions the fine tuning of stomatal conductance may require slow opening and closing reactions to avoid overshooting responses. Such a mechanism would also explain why our second expectation, a particularly fast loss of induction in the shade, was not fulfilled.

Given the observed slow reaction of CO_2 exchange to increased PFD during the induction experiments, it was expected that light flecks would be of little importance for the *in situ* carbon budgets in this epiphyte. Direct measurements of gas exchange in the natural understorey environment on BCI confirmed this expectation (Fig. 4; Table 2). In fact, the relationship between total PFD and diurnal carbon gain was negative. Although this trend was only marginally significant ($P = 0.07$), and is based on only five diel measurements, it differs entirely from the positive relationships normally observed between these two parameters (e.g. Pfitsch and Pearcy, 1989*a*). The reaction of CO_2 exchange

to light flecks was particularly slow in the afternoon. Similar differences in stomatal behaviour were found by Allen and Pearcy (2000), but the diurnal variation in g_w in the shrub *Psychotria limonensis* was not as pronounced as that in *Aspasia*: this orchid hardly reacted at all to light flecks in the afternoon (Fig. 4), which explains the large discrepancy between the measured diurnal carbon gain for 10 Mar. 1999 and the results of a model based on the measured light dependency of assimilation (Table 2). On average, however, diurnal carbon gain was 66 % of model predictions. All steady-state models overestimate *in situ* carbon gain in highly dynamic light environments (Stegemann *et al*., 1999), but the difference allows us to quantify the 'costs of low induction'. Remarkably, the average discrepancy between measurement and model is quite similar to that found by Pfitsch and Pearcy (1989*a*) in a study of the redwood forest understorey herb, *Adenocaulon bicolor*.

All the measurements discussed above were conducted on well-watered plants. However, even in the wet season on BCI there are rainless periods lasting many hours or even a few days (Windsor, 1990). Therefore, it was essential to study the effect of drought on leaf gas exchange. Surprisingly, the absence of irrigation had almost no effect on induction kinetics during the first few days (Fig. 5). Even after 6 d of drought *Aspasia* reached 60 % of initial NP_{max} and 55 % of initial g_{max}, and showed no delay in induction time. On the subsequent day, however, gas exchange was finally severely reduced and it virtually ceased on day 8. This suggests that the bark epiphyte *Aspasia principissa* is capable of maintaining a rather constant level of carbon gain during the wet season, when rainless periods of more than a few days are rare (Windsor, 1990). In the dry season, however, gas exchange is expected to be restricted to a few days after the occasional rain, as is the case for epiphytes in more exposed sites of the upper canopy (Zotz and Winter, 1994).

In conclusion, vascular epiphytes in moist lowland forests, even those growing in the understorey, face frequent drought stress, and water conservation should take priority over carbon gain. Consistent with this expectation, light flecks had a rather limited impact on leaf CO_2 budgets in *Aspasia principissa*. We are currently investigating how these results at the leaf level scale up to growth of entire plants. First results indicate that relative growth rates in *Aspasia principissa* are very low indeed (Schmidt and Zotz, 2002).

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