Physiologia Plantarum
An International Journal for Plant Biology

Physiologia Plantarum 162: 506–517. 2018 ISSN 0031-9317

Acclimation of photosynthesis to lightflecks in tomato leaves: interaction with progressive shading in a growing canopy

Elias Kaiser[a,*], Shizue Matsubara[b], Jeremy Harbinson[a], Ep Heuvelink[a] and Leo F.M. Marcelis[a]

[a]Horticulture and Product Physiology, Department of Plant Sciences, Wageningen University, Wageningen, the Netherlands
[b]IGB-2: Pflanzenwissenschaften, Jülich, Germany

Correspondence
*Corresponding author,
e-mail: elias.kaiser@wur.nl

Received 18 August 2017;
revised 20 October 2017

doi:10.1111/ppl.12668

Plants in natural environments are often exposed to fluctuations in light intensity, and leaf-level acclimation to light may be affected by those fluctuations. Concurrently, leaves acclimated to a given light climate can become progressively shaded as new leaves emerge and grow above them. Acclimation to shade alters characteristics such as photosynthetic capacity. To investigate the interaction of fluctuating light and progressive shading, we exposed three-week old tomato (*Solanum lycopersicum*) plants to either lightflecks or constant light intensities. Lightflecks of 20 s length and 1000 µmol m^{-2} s^{-1} peak intensity were applied every 5 min for 16 h per day, for 3 weeks. Lightfleck and constant light treatments received identical daily light sums (15.2 mol m^{-2} day^{-1}). Photosynthesis was monitored in leaves 2 and 4 (counting from the bottom) during canopy development throughout the experiment. Several dynamic and steady-state characteristics of photosynthesis became enhanced by fluctuating light when leaves were partially shaded by the upper canopy, but much less so when they were fully exposed to lightflecks. This was the case for CO_2-saturated photosynthesis rates in leaves 2 and 4 growing under lightflecks 14 days into the treatment period. Also, leaf 2 of plants in the lightfleck treatment showed significantly faster rates of photosynthetic induction when exposed to a stepwise change in light intensity on day 15. As the plants grew larger and these leaves became increasingly shaded, acclimation of leaf-level photosynthesis to lightflecks disappeared. These results highlight continuous acclimation of leaf photosynthesis to changing light conditions inside developing canopies.

Introduction

Acclimation of a plant to a new light environment is characterized by changes in its phenotype (environmental plasticity), and is constrained by the plant's genetics. Acclimation to a given light intensity may include changes in the quantity and stoichiometry of components such as chlorophylls *a* and *b*, Rubisco, the cytochrome b_6/f complex, light harvesting complexes of photosystem II, plastocyanin and ATPase, the number of chloroplasts per cell and (irreversibly) stomatal and vein density (Rascher and Nedbal 2006, Schöttler and Tóth 2014,

Abbreviations – *A*, net photosynthesis rate; A_{24}, daily leaf carbon budget; A_{avg}, average *A* during the photoperiod; A_{max}, CO_2- and light-saturated *A*; A_{sat}, light-saturated *A*; C_i, substomatal CO_2 partial pressure; F_m', maximum fluorescence on light-adapted leaves; F_s, fluorescence emission in a leaf adapted to actinic light; g_s, stomatal conductance; LA, leaf area; PAR, photosynthetically active radiation; R, leaf respiration in darkness; t_{A50}, time to reach 50% of full photosynthetic induction; t_{A90}, time to reach 90% of full photosynthetic induction; t_{gs50}, time to reach 50% of final g_s; t_{gs90}, time to reach 90% of final g_s; WUE_i, intrinsic water use efficiency; Φ_{PSII}, efficiency of electron transport through photosystem II.

Retkute et al. 2015). Light intensity often fluctuates in natural environments in the order of seconds to minutes (Pearcy et al. 1990). Acclimation to fluctuating light depends on the duration, intensity and frequency of the lightflecks applied (Walters 2005, Alter et al. 2012, Retkute et al. 2015): fewer, longer lightflecks seem to enhance the acclimatory response (Yin and Johnson 2000, Walters 2005, Alter et al. 2012). The acclimatory response may result in increased light-saturated net photosynthetic rate (A_{sat} [μmol m^{-2} s^{-1}]; Wayne and Bazzaz 1993, Watling et al. 1997, Leakey et al. 2003, Zheng et al. 2006, Blom and Zheng 2009, Retkute et al. 2015) or increased photosynthetic rate under conditions of both light- and CO_2-saturation (A_{max}; Yin and Johnson 2000, Zheng et al. 2006). Apart from the steady-state characteristics of photosynthesis, the dynamic properties of photosynthesis might also change during acclimation to fluctuating light. This would mean that in a leaf acclimated to fluctuating light, net photosynthesis rates (A) or stomatal conductance (g_s) would change more quickly after a change in light intensity than in a leaf that is not acclimated to fluctuating light. In other words, the kinetic properties of assimilation could be 'trained' to respond more quickly to light intensity fluctuations. Several studies have investigated this possibility, but have found no such 'training' effect (Sims and Pearcy 1993, Ernstsen et al. 1997, Leakey et al. 2003, Kubasek et al. 2013).

In previous studies using multiple species, some species showed enhanced A_{sat} or A_{max} when growing under fluctuating light, while others did not (Wayne and Bazzaz 1993, Watling et al. 1997, Yin and Johnson 2000, Blom and Zheng 2009, Vialet-Chabrand et al. 2017). This apparent species specificity may be explained by differences in the maximum extent or the rate of acclimation to fluctuating light. In *Arabidopsis thaliana*, e.g. the increase of A_{max} after a transition from a low- to a high-light intensity took approximately 3–7 days, depending on the genotype (Athanasiou et al. 2010, van Rooijen et al. 2015). Furthermore, it is not clear how canopy growth and the resulting shading of the leaf influence the leaf-level acclimation to fluctuating light. To investigate this, we exposed young tomato plants to either lightflecks or constant light intensities and followed the steady-state and dynamic characteristics of photosynthesis of several leaves during canopy development over the course of 3 weeks.

Materials and methods

Plant material and growth conditions

Solanum lycopersium cv. Cappricia (Rijk Zwaan, De Lier, the Netherlands) plants were grown in a climate chamber using 4-l pots and soil (ED73, Einheitserde, Sinntal-Altengronau, Germany) enriched with nutrients (0.25% m/v fertilizer salts, pH 5.8). Plants were fed with nutrient solution once a week (1:100 dilution, 20–5–10–2 N–P–K–Mg; Hakaphos, Scotts, OH), and watered as needed. Conditions in the climate chamber were: 300 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) provided by a mixture (50% each) of mercury (Master HPI-T Plus, 400 W; Philips, Eindhoven, the Netherlands) and sodium vapor high pressure lamps (SON-T Agro, 400 W; Philips), 16 h photoperiod, 22/20°C day/night, 450 μbar CO_2 partial pressure and 70% relative humidity.

Treatments

When plants were 23 days old, eight plants were exposed to flecked light ('lightfleck'), while another eight plants were exposed to constant light ('control'). Apart from the light treatments, environmental conditions were identical as described above. Plants were standing in two rows of four plants each; on a table that was 127 cm long and 55.5 cm wide. Pot diameter was 21 cm, and the distance between pots was 5 cm in rows and between rows (one plant per pot). Lightflecks were applied by moving an array of five lamps over the plants from one side to another. These lamps (type 46 900, Schwabe, Eutingen im Gäu, Germany) were rectangular (10.5 × 8.7 cm) and each lamp was comprised of 12 SMD LED chips (Samsung, Seoul, South Korea). The light color of the lamps was 6400 K. The total array was 43.5 cm wide, 10.5 cm long and was comprised of 15 × 4 LED chips. During each lightfleck, the light intensity was increased above the background light intensity for approximately 20 s, every 5 min during the photoperiod (192 lightflecks per day). Peak light intensity during lightflecks was approximately 1000 μmol m^{-2} s^{-1} at the highest part of the canopy (20 cm below the lamps), while average light intensity during lightflecks was approximately 650 μmol m^{-2} s^{-1} (Fig. 1A). As the array moved back to its rest position with the LEDs off, it produced a 'shadefleck', which reduced the overall light intensity received in the lightfleck treatment (Fig. 1A). The spectra of the LEDs had peaks at 450 and 540 nm (Fig. 1B). Plants in treatments were adjusted daily as follows: (1) the position of every plant within the treatment was changed in a randomized fashion, (2) the distance of the tallest part of a plant to its light source was changed several times per day, as the highest point in the canopy changed due to plant growth and leaf movement. Each pot was placed on a small platform (Swiss Boy lab jacks, Sigma Aldrich, St. Louis, MO) to adjust the distance between the LEDs and the growing shoot tip individually. Average light intensity

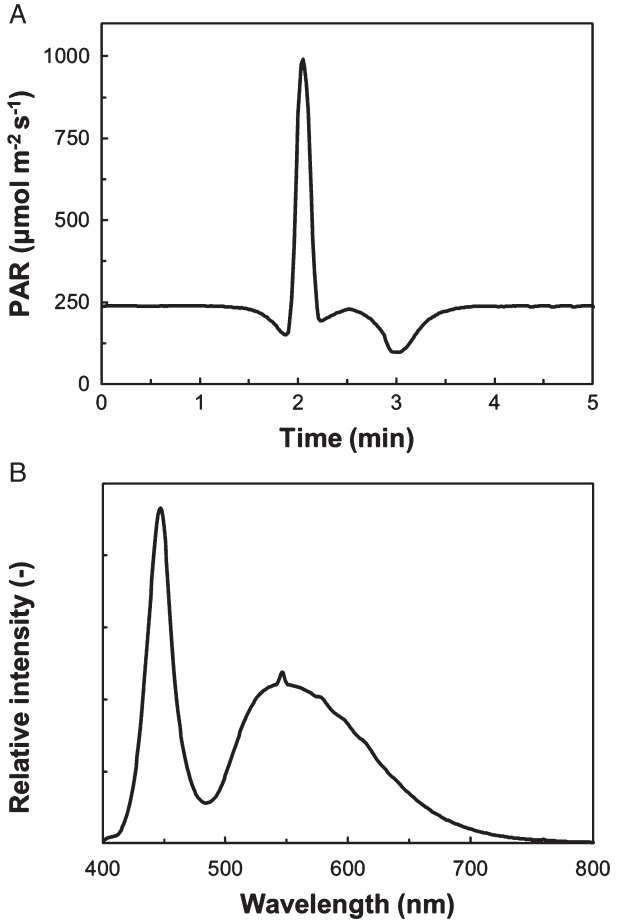

Fig. 1. Characteristics of the lightfleck treatment. (A) time course of photosynthetically active radiation (PAR) during a lightfleck cycle, measured 0.2 m below the LED array. (B) spectral output of the LED lamps used to produce the lightflecks.

in both treatment groups was 264 ± 8 µmol m^{-2} s^{-1} PAR (mean ± standard error of means, SEM) at the tip of the plants, and great care was taken so that both treatment groups received the same light sum every day (15.2 mol m^{-2} day^{-1}). In the lightfleck treatment, 84% of the daily light sum was supplied by the background light (i.e. the remaining 16% was supplied by lightflecks), suggesting that plants under both treatments received roughly the same light spectrum. Light intensity in the treatments was measured regularly using the same data logger and point sensor (LI-189 light meter, Li-Cor Biosciences, Lincoln, MO).

Measurements

Gas exchange measurements – All gas exchange measurements were performed with the LI-6400 photosynthesis system (Li-Cor Biosciences, Lincoln, MO), equipped with the fluorescence chamber (Li-6400-40, area: 2 cm^2). The light in the fluorescence chamber was supplied by a mixture (90/10%) of red and blue LEDs. The intensity of red LEDs peaked at 635 nm, that of blue LEDs at 465 nm; the bandwidth of half-maximum intensity was 10 nm in both LED types.

Lightfleck responses – To test the reaction of leaves to lightflecks, the fluorescence chamber was clamped on the leaves and they were adapted to 250 µmol PAR m^{-2} s^{-1} until g_s was stable (30–90 min). Then, leaves were exposed to lightflecks of 20 s duration and 1000 µmol PAR m^{-2} s^{-1} once every 5 min for 60 min, to mimic the lightfleck environment experienced under the lightfleck treatment. Conditions in the cuvette were: 400 ± 3 µbar CO_2 partial pressure, 22 ± 0.2°C cuvette temperature, 70 ± 3% relative humidity and 500 ± 1 mol s^{-1} flow rate of air through the cuvette.

Photosynthetic induction – To assess the rate of change of A and g_s after a step change in light intensity, leaves were dark-adapted until g_s was steady (60–120 min). Then, the light intensity was increased to 1000 µmol m^{-2} s^{-1}, and data were logged every second for 60 min. Other conditions were as described in 'lightfleck responses'. The times to reach 50 and 90% of photosynthetic induction (t_{A50}, t_{A90}) and the times to reach 50 and 90% of final g_s (t_{gs50}, t_{gs90}) were calculated as in Kaiser et al. (2017).

Diurnal photosynthesis – To follow the diurnal trends of light interception, A, g_s, and intrinsic water use efficiency (WUE_i, A g_s^{-1}) of plants in their respective treatments, gas exchange of leaves of several layers in the canopy was measured several times per day. For these measurements, the upper part of the leaf chamber fluorometer was removed, while the cuvette was still intact and sealed with a transparent window (2 cm^2). Measurements were logged once per second. In the lightfleck treatment it was ensured that a whole lightfleck sequence, including the period between lightflecks, was captured in each case, and the average of the 5 min sequence was calculated. In the control treatment, averages of 2 min were used. Other conditions were as described in 'lightfleck responses'.

A/C_i curves – To compare the response of A to different substomatal CO_2 partial pressures (C_i), leaves were adapted to 500 µbar CO_2 and 1000 µmol PAR m^{-2} s^{-1} for 30 min. Then, leaves were exposed to a range of CO_2 partial pressures between 50 and 1500 µbar. At each step in CO_2 partial pressure, after reaching steady-state A, data were logged every 5 s, and an average of 1 min was used to calculate A and C_i. Additionally, to assess the relationship between C_i and the efficiency of electron transport through photosystem II (Φ_{PSII}), a saturating flash was applied at each CO_2 partial pressure

and chlorophyll fluorescence parameters F_m' (maximum fluorescence on light-adapted leaves) and F_s (fluorescence emission in a leaf adapted to actinic light) were recorded. Φ_{PSII} was calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$. Saturating flashes were applied using a multiphase flash protocol (Loriaux et al. 2013) consisting of three phases, with 7400–7800 µmol m^{-2} s^{-1} maximum flash intensity, 60% decrease of flash intensity during the second phase (60% ramp) and 0.3, 0.7, and 0.4 s duration for the three phases, respectively. Measuring light intensity was approx. 1 µmol m^{-2} s^{-1}. Other conditions were as described in 'lightfleck responses'.

Leaf area growth – To follow the growth of whole-shoot leaf area over time, length and width of single leaves was measured using a generic flexible ruler. These measurements were performed between 10:00. and 12:00. An empirical linear relationship (slope = 0.3493, $R^2 = 0.96$) between leaf area and leaf length × width was obtained by destructively measuring leaf area (using the LI-3100 leaf area meter, Li-Cor Biosciences). These measurements were performed on additional plants that did not belong to any of the treatment groups but had grown under the same conditions.

Biomass and leaf area – Shoots were harvested after the end of the treatment, split into leaves and stems and weighed. Leaf area was determined using the LI-3100 leaf area meter. After drying samples at 85°C for 13 days, dry weights of leaves and stems were determined.

Calculations

The daily leaf carbon budget (A_{24}; mmol day^{-1}) was determined for leaf 2 on days 2, 5, 9, 12 and 16, and for leaf 4 on days 5, 9, 12 and 16, as:

$$A_{24} = \left(\left(A_{avg} \times 16\right) - (R \times 8)\right) \times (3600/1000) \times LA \quad (1)$$

where A_{avg} (µmol m^{-2} s^{-1}) is the average of the net leaf photosynthesis rate of five point measurements spread over approximately 8 h during the photoperiod. R (µmol m^{-2} s^{-1}) is leaf respiration in darkness, and was determined as net photosynthesis rate in leaves that had been dark-adapted for 60–120 min. LA (m^2) is the area of the leaf. A_{avg} and R were multiplied by the duration of the light (16 h) and dark (8 h) periods, respectively, and values were scaled from rate per second to rate per hour (3600) and from µmol to mmol (1000). R was determined on days 4, 11, and 18, and linear interpolation was used to determine its values on the desired dates. Because no significant difference was found for R between treatments, the same values were assumed for lightfleck and control leaves. LA was measured on

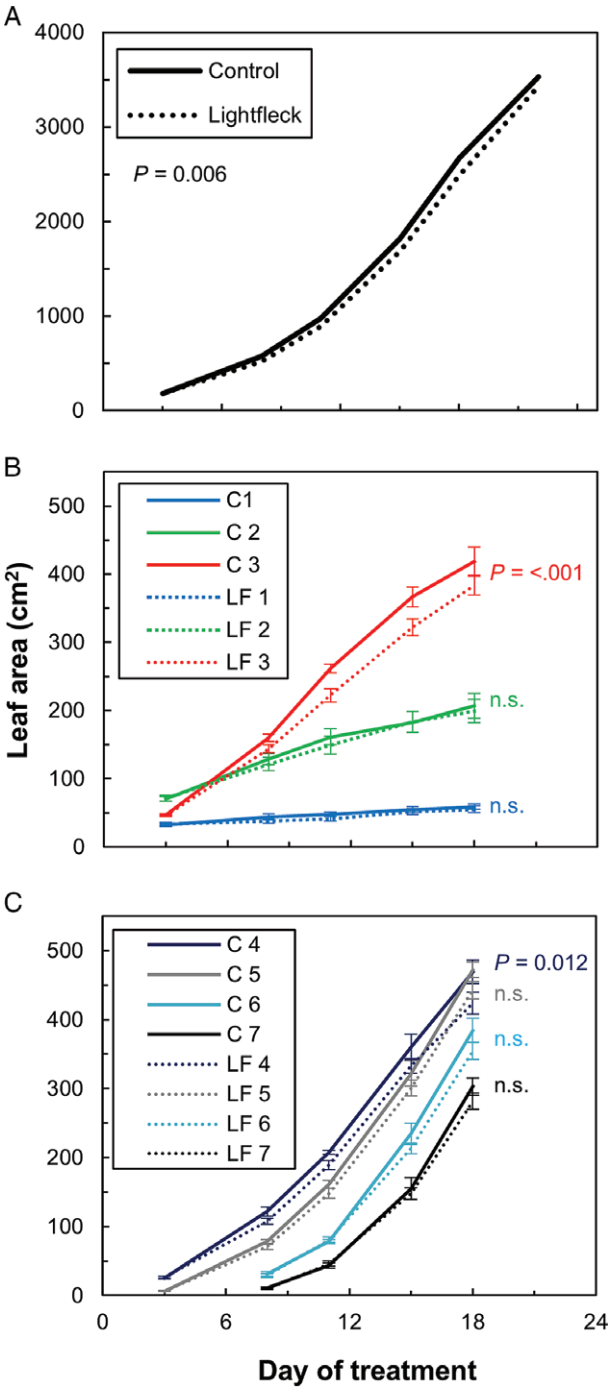

Fig. 2. Leaf area growth under constant (control, C; solid lines) or flecked light (lightfleck, LF; dotted lines). (A) Total leaf area per tomato plant. (B) Area of individual leaves 1, 2, and 3 (counting from below). (C) Area of leaves 4, 5, 6, and 7. *P*-values denote significant treatment effects on total leaf area (panel A) and leaf area of single leaves (panels B and C). n.s. denotes absence of significant treatment effects on area of single leaves. Averages ± SEM, n = 7–8.

Table 1. Growth parameters of aboveground plant parts. Tomato plants were grown for 3 weeks under constant (control) or flecked light (lightfleck) and then harvested destructively. Numbers denote average ± SEM, n = 7–8. *P*-values of two-sided student's *t*-tests or Mann–Whitney U-tests are shown; values that show a significant difference ($P < 0.05$) are shown in bold. [a]Data have been analyzed using nonparametric Mann–Whitney U-test.

		Treatment		
	Growth parameter	Control	Lightfleck	*P*-value
Leaf	Fresh weight (g)	207.1 ± 6.2	191.4 ± 7.2	0.125
	Dry weight (g)	16.7 ± 0.7	15.8 ± 0.6	0.341
	Area (m^2)	0.44 ± 0.01	0.42 ± 0.01	0.263
	Specific leaf area ($cm^2\ g^{-1}$)	21.4 ± 0.1	22.1 ± 0.3	**0.040**[a]
	Dry matter content (%)	8.1 ± 0.1	8.3 ± 0.2	0.536[a]
Stem	Fresh weight (g)	51.8 ± 0.5	45.0 ± 2.1	**0.014**[a]
	Dry weight (g)	2.96 ± 0.05	2.52 ± 0.13	**0.014**[a]
	Dry matter content (%)	5.71 ± 0.06	5.60 ± 0.15	0.955[a]
Whole shoot	Fresh weight (g)	259 ± 6	240 ± 8	0.079
	Dry weight (g)	19.7 ± 0.7	18.3 ± 0.7	0.206

days 3, 8, 11, 15, and 18 (data in Fig. 2); second degree polynomials ($R^2 > 0.99$) were used to determine its values on the desired dates.

Statistical tests

Most values are shown as average ± SEM. In order to detect significant differences between treatments, data were first tested for homogeneity of variances (Bartlett's test) and for normal distribution (Shapiro–Wilk test). Values that were measured repeatedly on the same experimental units were tested using analysis of variances (ANOVA), and day of measurement was included as a treatment factor. For values that were determined once, a two-sided student's *t*-test was conducted. Datasets that violated the assumptions for ANOVA or *t*-test were log-transformed. If data after log-transformation still violated the necessary assumptions, then the nonparametric Kruskal–Wallis ANOVA (instead of ANOVA) or Mann–Whitney U-test (instead of the *t*-test) was performed on the original data. All statistical tests were performed using Genstat 18th edition (VSN International, Hempstead, UK).

Results

Growth of plants under constant and flecked light

Stems of plants growing under flecked light (lightfleck) weighed less than stems of plants growing under constant light (control), both in fresh ($P = 0.01$) and dry weight ($P = 0.01$; Table 1). Also, leaves of plants growing under flecked light were significantly thinner (larger SLA; $P = 0.04$) than those of control plants (Table 1). Throughout the experiment, lightfleck plants displayed a significantly smaller total leaf area than control plants ($P = 0.006$; Fig. 2A). Furthermore, several leaves that were high in the canopy in the early stages of the experiment (leaves 3 and 4, counting from the bottom) had a significantly smaller leaf area in lightfleck plants (Fig. 2B, C). The interaction term between day of measurement and treatment was not significant for either total leaf area ($P = 0.583$) or area of leaves 3 ($P = 0.443$) and 4 ($P = 0.553$), indicating that leaf area was significantly affected by the lightfleck treatment throughout the complete period of the experiment. However, total leaf area was not significantly different at the final harvest after 22 days (Table 1). Hence, with progressive canopy closure, the initial difference between treatments disappeared.

Plasticity of acclimation to flecked light

In order to follow single leaves through their life cycle, we monitored photosynthetic characteristics of the 2nd and 4th leaves (counting from the bottom). Since both leaf ages showed similar responses to the two treatments, only data depicting the behavior of leaf 4 are shown here; the photosynthetic responses of leaf 2 are shown in Figs S1–S3 and S7 (Supporting information) and Table 2.

During progressive shading by other leaves, the photosynthetic capacity (measured as the steady-state response of photosynthesis, *A*, to substomatal CO_2 partial pressure, C_i) of leaves 2 and 4 slowly decreased in both treatments (Fig 3 and Fig. S1). An ANOVA of the response of *A* or Φ_{PSII} to the highest C_i value, representing the leaf's photosynthetic capacity, revealed a significant interaction between day of measurement and treatment ($P < 0.05$) in both leaf 4 and 2, for *A* as well as Φ_{PSII}. Thus, in leaf 4, both *A* and Φ_{PSII} were unaffected on day 7 (Fig. 3A) while on day 14, both were significantly enhanced in leaves under the lightfleck treatment (Fig. 3B). On day 20, Φ_{PSII} remained significantly

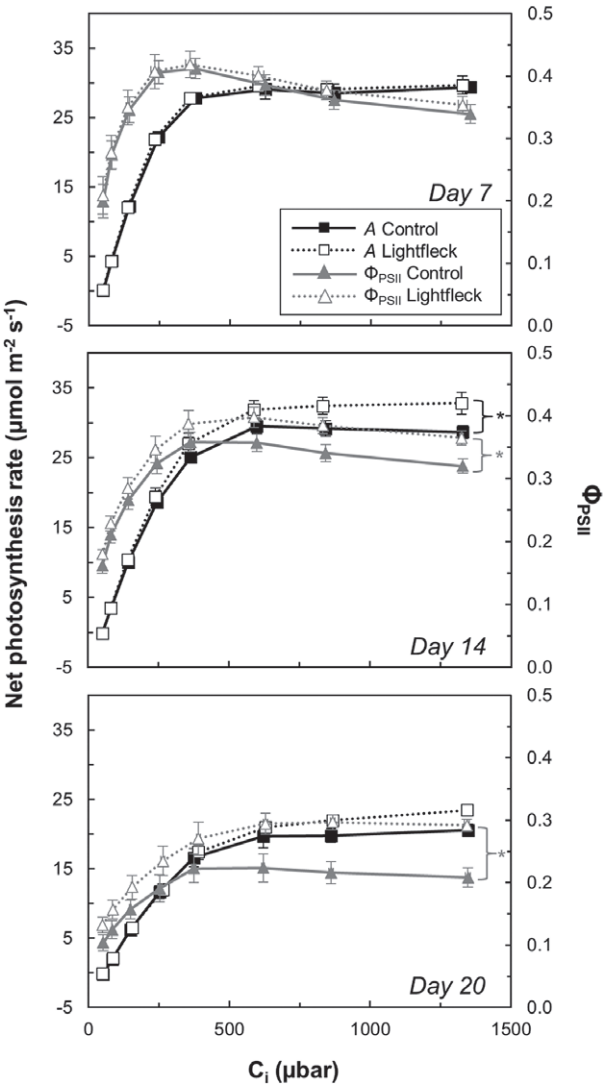

Fig. 3. Response of net photosynthesis rate (A, black symbols) and Φ_{PSII} (gray symbols) to substomatal CO_2 partial pressure (C_i) in leaf 4 (counting from below) of tomato plants growing under constant (control; closed symbols, solid lines) or flecked light (lightfleck; open symbols, dotted lines) on different days during the treatment. Black (gray) stars denote significant ($P < 0.05$) treatment effects on A (Φ_{PSII}) at highest C_i on a given day of measurement. Symbols denote averages ± SEM, n = 3.

enhanced in lightfleck leaves while A was not significantly different (Fig. 3C). Similarly, in leaf 2, both A and Φ_{PSII} were not significantly different on days 0 and 7 of the experiment (Fig. S1A, B). However, on day 14, CO_2-saturated A and Φ_{PSII} were significantly higher in plants growing under flecked light (Fig. S1C), but that difference disappeared on day 20 (Fig. S1D).

In contrast to the steady-state photosynthetic capacity assessed by A/C_i (or Φ_{PSII}/C_i) curves, repeated measurements of diurnal variations in A, g_s, C_i and WUE_i revealed no systematic differences between leaf 4 of plants growing under constant or flecked light (Fig. 4). Data from leaf 2 confirmed that impression (Fig. S2). While the incident PAR on leaf 4 was slightly but consistently higher on days 5–12 in the lightfleck treatment, on day 16 it had decreased in both treatments to the same level (Fig. 4D), indicating that leaf 4 was becoming overgrown by leaves higher up in the canopy. We additionally tested whether differences in diurnal gas exchange characteristics could be discerned in young leaves that had grown and developed under the two treatments: For this, half of the plants were swapped between treatments on the last day of the experiment (day 21) and A, g_s and light-use efficiency were determined in leaf 9 in each of the four groups (i.e. plants grown under constant light were measured under constant and flecked light, as were plants grown under flecked light). The results clearly showed that there were no effects of the growth light treatments on A, g_s or light-use efficiency in these leaves (Fig. S3).

To closely examine the photosynthetic properties under dynamic light conditions, gas exchange characteristics were measured during a series of lightflecks. Across the 3 days of measurement (days 6, 13, and 19), A was significantly larger both during ($P = 0.021$) and between lightflecks ($P = 0.041$; Fig. 5A, C, E). Additionally, A during lightflecks was significantly lower ($P = 0.018$) on day 13, compared to responses on days 6 and 19, which were not significantly different from one another; A between lightflecks was not significantly affected by day of measurement. Treatment effects on A were not accompanied by corresponding changes in g_s, i.e. g_s was not significantly affected by the lightfleck treatment (or by day of measurement) on either of the days of measurement (Fig. 5B, D, F). Detailed time courses of the responses of A and g_s during these lightfleck measurements can be found in Figs S4–S6. In leaf 2, no treatment effects were detected, either on A or g_s (Fig. S7). No significant interaction between treatment and day of measurement was found for either A or g_s, both in leaf 4 and 2.

When dark-adapted leaves were exposed to a step change in light intensity (1000 µmol m^{-2} s^{-1}), there were no differences between the treatments in steady-state A at 0 and 1000 µmol m^{-2} s^{-1} on either day 15 or 18 (data not shown). On day 15 of the experiment, however, leaf 2 under the lightfleck treatment showed a significantly shorter time to reach 90% of the final steady-state A (Table 2). This difference in the rate of photosynthetic induction was not caused by faster stomatal opening (Table 2), larger initial g_s ($P = 0.81$), different initial A ($P = 0.77$), or different final A ($P = 0.75$). No differences in induction kinetics of A or stomatal opening were seen in leaf 4 (measured on day 18, Table 2).

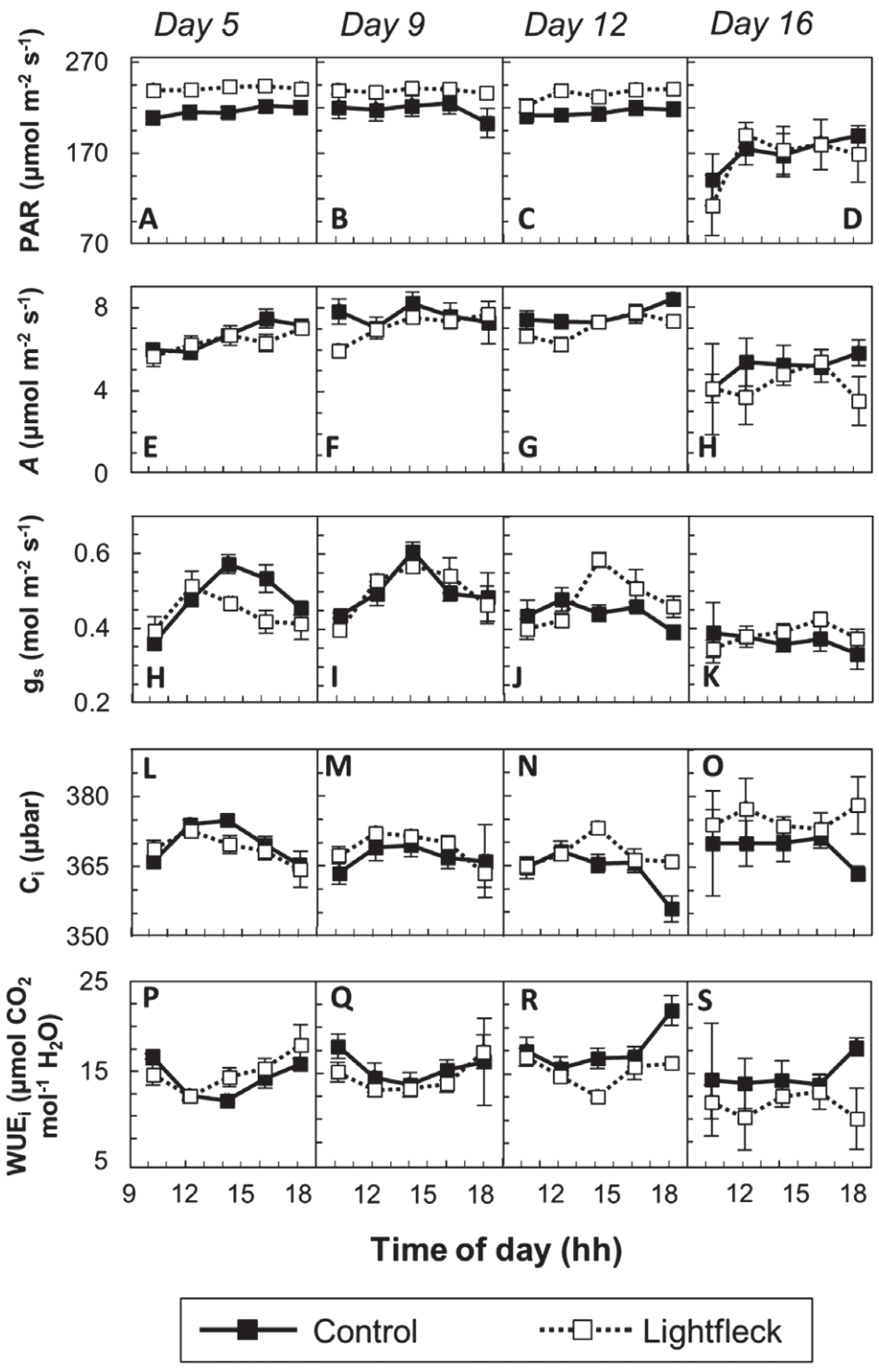

Fig. 4. Diurnal changes of incident photosynthetically active radiation (PAR; A–D), net photosynthesis rate (*A*; E–H), stomatal conductance (g_s; H–K), substomatal CO_2 partial pressure (C_i; L–O) and intrinsic water use efficiency (WUE_i; P–S) in leaf 4 (counting from below) of tomato plants growing under constant (control; closed symbols, solid lines) or flecked light (lightfleck; open symbols, dotted lines) on different days during the treatment. Symbols denote hourly averages ± SEM, n = 4–5.

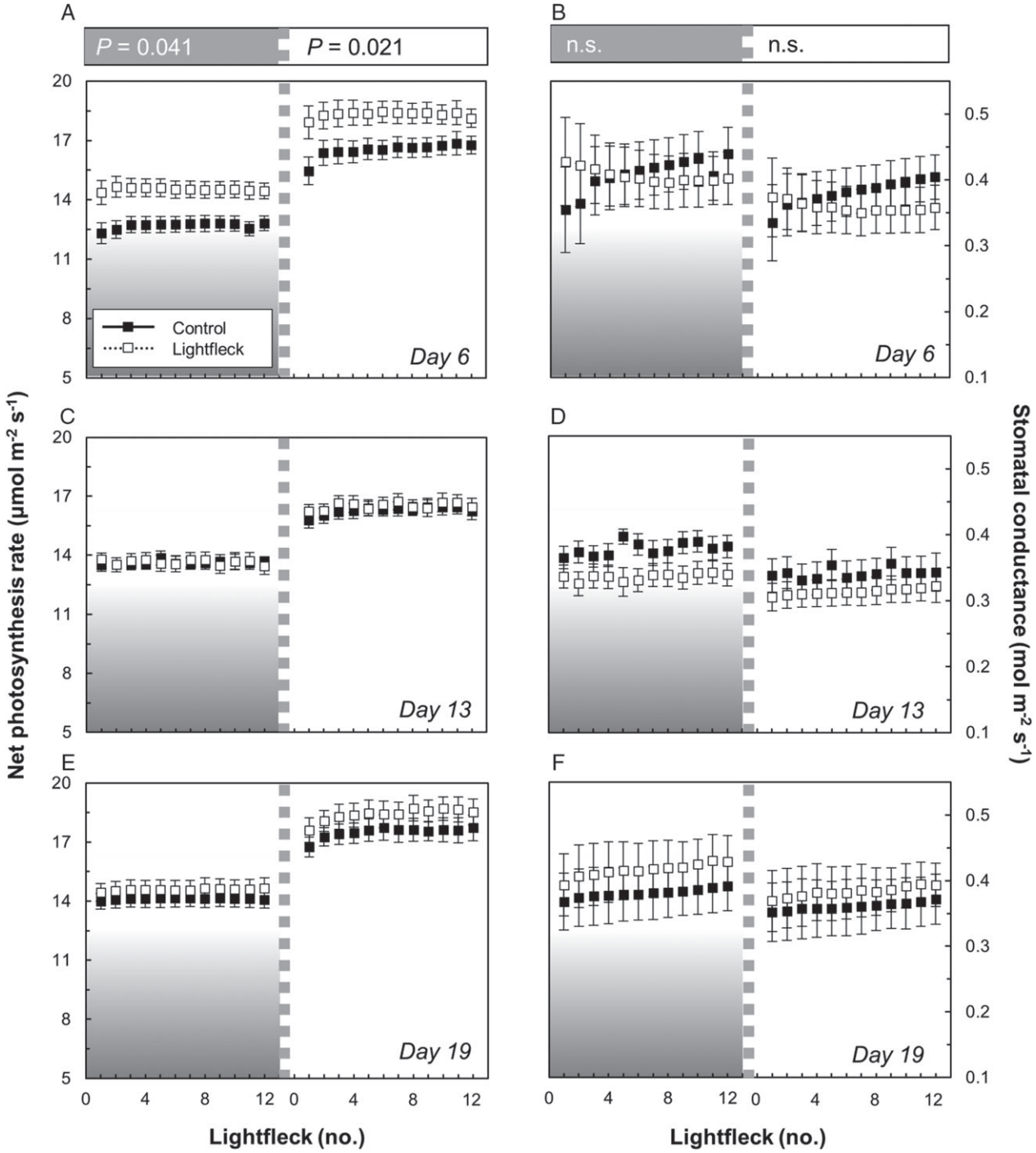

Fig. 5. Response of net photosynthesis rate (*A*) and stomatal conductance (g_s) of leaf 4 (counting from below) of plants grown under constant (control, closed symbols) or flecked light (lightfleck, open symbols) to a series of lightflecks (1000 µmol m^{-2} s^{-1} for 20 s) in a lower background light intensity (250 µmol m^{-2} s^{-1} for 280 s), on different days during the treatment. During measurements, leaves were exposed to lightflecks produced in the gas exchange cuvette. Rates were averaged for the time between lightflecks (shaded area on the left side of figures) and for the duration of each lightfleck (right side of figures). Bars above panel A indicate significant treatment effects on *A*, both during and between lightflecks, on all 3 days of measurement; bars above panel B indicate absence of significant treatment effects on g_s. Symbols denote mean ± SEM, n = 4–5.

Table 2. Time (min) to reach 50 or 90% of final net photosynthesis rates (t_{A50} or t_{A90}, respectively) and time to reach 50 or 90% of final stomatal conductance (t_{gs50} or t_{gs90}, respectively) after a stepwise increase in light intensity from 0 to 1000 µmol m^{-2} s^{-1} in dark-adapted leaves. Measurements were conducted on day 15 of the lightfleck experiment in leaf 2 and on day 18 in leaf 4. Numbers denote mean ± SEM, n = 4. *P*-values of two-sided student's *t*-tests are shown; values that show a significant difference ($P < 0.05$) are shown in bold.

		Treatment		
Leaf no.	Parameter	Control	Lightfleck	*P*-value
2	t_{A50}	2.6 ± 0.2	2.1 ± 0.4	0.361
	t_{A90}	27.2 ± 1.2	21.8 ± 0.8	**0.010**
	t_{gs50}	27.3 ± 1.1	27.1 ± 0.6	0.872
	t_{gs90}	48.9 ± 0.4	48.7 ± 0.9	0.841
4	t_{A50}	2.5 ± 0.2	2.1 ± 0.5	0.484
	t_{A90}	21.8 ± 1.6	20.4 ± 0.9	0.479
	t_{gs50}	23.9 ± 2.2	24.9 ± 2.0	0.754
	t_{gs90}	47.6 ± 1.4	49.2 ± 2.7	0.624

Carbon budgets of plants growing under flecked and constant light

Differences in whole-plant light-use efficiency was determined by calculating the daily leaf carbon budget of leaves 2 and 4. Leaves growing under fluctuating light used the light they received on average 17 and 22% less efficiently than leaves growing under constant light (Tables 3 and S1). This difference was caused by both lower *A* on an area basis (Figs 4, S2) as well as lower light capture due to smaller leaves (Fig. 2 B, C). In leaf 2, the negative effects of the lightfleck treatment on daily leaf carbon budget almost completely diminished as the leaves became more strongly shaded (days 12 and 16; Fig. S2); they acquired only 1.8% less carbon on those days than the control leaves (Table S1).

Discussion

Plants grow more slowly under fluctuating light: limitations to light-use efficiency

Plants growing under flecked light used the light less efficiently, as evidenced by lower stem weight (Table 1) and smaller leaf area during growth (Fig. 2). The finding of a lower light-use efficiency in fluctuating light (Tables 3, S1) despite identical light sums is in agreement with previous reports from similar experiments (Watling et al. 1997, Leakey et al. 2003, Zheng et al. 2006, Alter et al. 2012, Kubasek et al. 2013, Vialet-Chabrand et al. 2017). After a stepwise increase in light intensity, the increase in *A* will respond with a delay, because increases in (1) the pool size of metabolites in the Calvin cycle, (2) the activation state of Calvin cycle enzymes, and (3) g_s do not respond instantaneously but with their own, characteristic delays (reviewed in Pearcy et al. 1996, Kaiser et al. 2015). After decreases in light intensity, a surplus of metabolites will keep *A* at a transiently higher rate than its steady-state value at low irradiance (post-illumination CO_2 fixation; Sharkey et al. 1986). However, this transient increase in *A* can be diminished by (1) the post-illumination CO_2 burst, which is caused by enhanced rates of respiration of phosphoglycolate (Vines et al. 1983) and (2) transiently reduced electron transport rates due to relatively slow relaxation of non-photochemical quenching (Armbruster et al. 2014, Kromdijk et al. 2016). Altogether, integrated *A* in a real leaf during and after a lightfleck is reduced compared to a hypothetical leaf whose *A* would respond instantaneously to changes in light intensity (Pearcy et al. 1985). Also, judging from the typical steady-state response of *A* to light intensity, the light intensities used for the constant light treatment (approximately 260 µmol m^{-2} s^{-1}) and for background light in the lightfleck treatment (approximately 250 µmol m^{-2} s^{-1}, Fig. 1A) are more efficiently used per quanta than the light intensity that was applied during the lightflecks (650 µmol m^{-2} s^{-1} on average). This means that even if *A* had reacted instantaneously to changes in light intensity, the light-use efficiency under lightflecks would still have been lower than under control conditions. The carbon gain of plants growing under lightflecks is therefore reduced because of transient reductions in *A* during a lightfleck, and because of the nonlinearity of the response of *A* to light intensity, compared to plants receiving the same light sum but at a constant light intensity.

Acclimation of leaf photosynthesis to lightflecks vs acclimation to shade

During this three-week experiment, we characterized various aspects of leaf gas exchange and chlorophyll fluorescence on a daily basis, i.e. responses of *A*, Φ_{PSII} and/or g_s to (1) CO_2 partial pressure in the steady-state, (2) series of lightflecks, (3) stepwise increases in light intensity after leaves had been dark-adapted, and (4) the light environment under which leaves were developing. Measurements were repeated several times during the day. Despite this broad and very frequent characterization, capturing the changes induced by the lightfleck treatment remained challenging.

Similar to previous reports on *A. thaliana* (Yin and Johnson 2000) and *Gerbera jamesonii* (Zheng et al. 2006), we found that after exposing plants to fluctuating light for several days, A_{max} increased in tomato leaves

Table 3. Daily leaf carbon budget of leaf 4 (mmol day^{-1}) of plants growing under fluctuating (lightfleck) or constant (control) light conditions, as calculated by Eqn. (1) on days 5, 9, 12, and 16 during the experiment.

		Day of experiment			
Parameter	Treatment	5	9	12	16
A_{avg} (μmol m^{-2} s^{-1})	Control	6.6	7.6	7.6	5.1
	Lightfleck	6.4	7.1	7.1	4.3
R (μmol m^{-2} s^{-1})	Both treatments	1.9	2.0	1.9	1.3
Leaf area (m^2)	Control	0.0060	0.0156	0.0249	0.0401
	Lightfleck	0.0056	0.0142	0.0226	0.0362
Daily leaf carbon budget (mmol day^{-1})	Control	1.95	5.93	9.59	10.30
	Lightfleck	1.73	4.99	7.94	7.55
Treatment effect on leaf C budget (%)		−13.1	−18.8	−20.8	−36.5

(Figs 3 and S1). It was recently shown that although A_{sat} was not significantly increased and A_{max} was significantly lower in *A. thaliana* leaves growing under fluctuating light, when both parameters were expressed per leaf mass instead of per leaf area they were both significantly larger under fluctuating light (Vialet-Chabrand et al. 2017). At the same time, leaves grown under fluctuating light intensities had equal or lower concentrations of photosynthetic proteins, suggesting that acclimation to fluctuating light enabled them to use these proteins more efficiently compared to leaves growing under constant light intensities (Vialet-Chabrand et al. 2017). Further, Vialet-Chabrand et al. (2017) reported significantly thinner leaves of plants grown under fluctuating light, which is consistent with the present and several other studies (Sims and Pearcy 1993, Wayne and Bazzaz 1993, Zheng et al. 2006, Kubasek et al. 2013).

Previous studies had consistently found that acclimation to fluctuating light did not entail a faster transient response of A or g_s to a step change in light intensity. These studies were conducted on widely differing species, such as basil (*Ocimum basilicum*) and the perennial *Impatiens wallerana* (Ernstsen et al. 1997), seedlings of the dipterocarps *Shorea leprosula* and *Hopea nervosa* (Leakey et al. 2003) and the understory shrub *Alocasia macrorrhiza* (Sims and Pearcy 1993). Unlike these studies, in the present work, leaf 2 of tomato plants from the lightfleck treatment displayed a significantly shorter time to reach 90% of full photosynthetic induction when subjected to a stepwise increase in light intensity after having been dark-adapted (on day 15 of the experiment, Table 2). Notably, this was found 1 day after a large increase in A_{max} was observed in the lightfleck treatment. The increase in t_{90} could not be explained by stomatal responses, such as faster stomatal opening or larger g_s before switching to high-light intensity (Kaiser et al. 2016). Considering that these differences were observed in leaves that were very likely partially shaded (on day 14, LAI of leaves above leaves 2 and 4 was approximately 1.5 and 0.8, respectively), this may indicate that lightflecks promote retention of the components that are required for rapid photosynthetic induction and the CO_2 response of photosynthesis, such as Rubisco activase.

Despite the changes in A_{max} and photosynthetic induction detected in the lightfleck plants, diurnal gas exchange measured in several leaves in the canopy did not indicate corresponding effects by the light treatment (Figs 4 and S3). One might expect stomata to open more widely under lightflecks than under constant light intensities, and therefore diurnal g_s to be larger throughout the day in the lightfleck treatment. However, this was clearly not the case. It might be that gas exchange under the prevailing light was not affected because the duration of lightflecks applied in this study was too short to fully elicit the responses observed in the steady state (Walters 2005).

Positive effects of lightflecks on partially shaded leaves: interaction with shade acclimation and nitrogen allocation?

In erect canopy stands such as tomato, light intensity decreases exponentially with canopy depth (Monsi and Saeki 2005). As light intensity is a strong driver for nitrogen content on a leaf area basis (Evans 1989, Anten et al. 1995, Hikosaka et al. 2016), leaf nitrogen content declines with progressive shading, which correlates with lower A_{max}, dark respiration, g_s, and earlier onset of senescence in shaded leaves (Pons and Pearcy 1994, Anten et al. 1995, Evans and Poorter 2001). In this way, plants maintain a nitrogen distribution such that whole-canopy carbon gains are very close to the theoretical optimum (Anten et al. 1995). A leaf which has acclimated to high-light intensities at the top of the canopy therefore has high nitrogen and protein contents per unit leaf area that

allow it to achieve high A_{max}, and it will keep those characteristics until it is overgrown and shaded by younger leaves (Pons and Pearcy 1994). Decreases in leaf nitrogen content can be observed quickly, e.g. within 3 days of shading in *Glycine max* (Pons and Pearcy 1994).

It can be hypothesized that a leaf that is partially shaded will retain leaf nitrogen contents when it is regularly exposed to lightflecks. Together with the leaf nitrogen, A_{max} would then be retained, as was seen after 14 days in our data (Figs 3B and S1). As lightflecks did not increase A_{max} in fully exposed leaves (as seen from measurements after 7 days, Fig. 3A), it may be that lightflecks were too short to lead to a high-light acclimation type response. Under partial shade, additional light provided by the transient lightflecks may have allowed retention of leaf nitrogen and high A_{max} by counteracting shade acclimation. After a longer exposure to deeper shade (e.g. after 20 days, when LAI above leaves 2 and 4 was approximately 3.1 and 2.0, respectively), probably the shade acclimation response prevailed over the effects of lightflecks (which would still be penetrating through the canopy) to maintain leaf nitrogen levels and A_{max} (Fig. 3C). More targeted research, including analysis of leaf nitrogen and protein contents, is required to investigate the possible role of lightflecks in leaf acclimation during canopy growth.

Conclusions

In conclusion, we show here that photosynthetic acclimation of leaves to lightflecks within a growing canopy is in itself a complex and continuous phenomenon. The positive effects of lightfleck acclimation on leaf gas exchange may be observed when leaves are partially shaded by younger leaves. In this situation, lightflecks may slow down shade acclimation and reduction of leaf nitrogen and protein levels. As leaves get increasingly shaded, the impact of acclimation to lightflecks disappears again.

Author contributions

All authors designed the study; E.K. performed data acquisition and analysis; E.K. and S.M. drafted the manuscript with input from all authors; all authors agreed on the final version of the manuscript.

Acknowledgements – This work was supported by the research programme of BioSolar Cells, co-financed by the Dutch Ministry of Economic Affairs. Additionally, this work was supported by Essent.

References

Alter P, Dreissen A, Luo FL, Matsubara S (2012) Acclimatory responses of *Arabidopsis* to fluctuating light environment: comparison of different sunfleck regimes and accessions. Photosynth Res 113: 221–237

Anten NPR, Schieving F, Werger MJA (1995) Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C_3 and C_4 mono-and dicotyledonous species. Oecologia 101: 504–513

Armbruster U, Carrillo LR, Venema K, Pavlovic L, Schmidtmann E, Kornfeld A, Jahns P, Berry JA, Kramer DM, Jonikas MC (2014) Ion antiport accelerates photosynthetic acclimation in fluctuating light environments. Nat Commun 5: 1–8

Athanasiou K, Dyson BC, Webster RE, Johnson GN (2010) Dynamic acclimation of photosynthesis increases plant fitness in changing environments. Plant Physiol 152: 366–373

Blom TJ, Zheng Y (2009) The response of plant growth and leaf gas exchange to the speed of lamp movement in a greenhouse. Sci Hortic 119: 188–192

Ernstsen J, Woodrow IE, Mott KA (1997) Responses of Rubisco activation and deactivation rates to variations in growth-light conditions. Photosynth Res 52: 117–125

Evans JR (1989) Photosynthesis and nitrogen relationship in leaves of C_3 plants. Oecologia 78: 9–19

Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ 24: 755–767

Hikosaka K, Anten NPR, Borjigidai A, Kamiyama C, Sakai H, Hasegawa T, Oikawa S, Iio A, Watanabe M, Koike T, Nishina K, Ito A (2016) A meta-analysis of leaf nitrogen distribution within plant canopies. Ann Bot 118: 239–247

Kaiser E, Morales A, Harbinson J, Kromdijk J, Heuvelink E, Marcelis LFM (2015) Dynamic photosynthesis in different environmental conditions. J Exp Bot 66: 2415–2426

Kaiser E, Morales A, Harbinson J, Heuvelink E, Prinzenberg AE, Marcelis LFM (2016) Metabolic and diffusional limitations of photosynthesis in fluctuating irradiance in *Arabidopsis thaliana*. Sci Rep 6: 1–13

Kaiser E, Kromdijk J, Harbinson J, Heuvelink E, Marcelis LFM (2017) Photosynthetic induction and its diffusional, carboxylation and electron transport processes as affected by CO_2 partial pressure, temperature, air humidity and blue irradiance. Ann Bot 119: 191–205

Kromdijk J, Glowacka K, Leonelli L, Gabilly ST, Iwai M, Niyogi KK, Long SP (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Science 354: 857–861

Kubasek J, Urban O, Santrucek J (2013) C_4 plants use fluctuating light less efficiently than do C_3 plants: a study

of growth, photosynthesis and carbon isotope discrimination. Physiol Plant 149: 528–539
Leakey ADB, Press MC, Scholes JD (2003) Patterns of dynamic irradiance affect the photosynthetic capacity and growth of dipterocarp tree seedlings. Oecologia 135: 184–193
Loriaux SD, Avenson TJ, Welles JM, McDermitt DK, Eckles RD, Riensche B, Genty B (2013) Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity. Plant Cell Environ 36: 1755–1570
Monsi M, Saeki T (2005) On the factor light in plant communities and its importance for matter production. Ann Bot 95: 549–567
Pearcy RW, Osteryoung K, Calkin HW (1985) Photosynthetic responses to dynamic light environments by Hawaiian trees. Plant Physiol 79: 896–902
Pearcy RW, Roden JS, Gamon JA (1990) Sunfleck dynamics in relation to canopy structure in a soybean (*Glycine max* (L.) Merr.) canopy. Agric For Met 52: 359–372
Pearcy RW, Krall JP, Sassenrath-Cole GF (1996) Photosynthesis in fluctuating light environments. In: Baker NR (ed) Photosynthesis and the Environment. Kluwer Academic, Dordrecht, pp 321–346
Pons TL, Pearcy RW (1994) Nitrogen reallocation and photosynthetic acclimation in response to partial shading in soybean plants. Physiol Plant 92: 636–644
Rascher U, Nedbal L (2006) Dynamics of photosynthesis in fluctuating light. Curr Op Plant Biol 9: 671–678
Retkute R, Smith-Unna SE, Smith RW, Burgess AJ, Jensen OE, Johnson GN, Preston SP, Murchie EH (2015) Exploiting heterogeneous environments: does photosynthetic acclimation optimize carbon gain in fluctuating light? J Exp Bot 66: 2437–2447
Schöttler MA, Tóth SZ (2014) Photosynthetic complex stoichiometry dynamics in higher plants: environmental acclimation and photosynthetic flux control. Front Plant Sci 5: 1–15
Sharkey TD, Seemann JR, Pearcy RW (1986) Contribution of metabolites of photosynthesis to postillumination CO_2 assimilation in response to lightflects. Plant Physiol 82: 1063–1068
Sims DA, Pearcy RW (1993) Sunfleck frequency and duration affects growth rate of the understorey plant, *Alocasia macrorrhiza*. Funct Ecol 7: 683–689
Vialet-Chabrand S, Matthews JSA, Simkin AJ, Raines CA, Lawson T (2017) Importance of fluctuations in light on pant photosynthetic acclimation. Plant Physiol 173: 2163–2179
Vines HM, ZP T, Armitage AM, Chen SS, Black CCJ (1983) Environmental responses of the post-lower illumination CO_2 burst as related to leaf photorespiration. Plant Physiol 73: 25–30
van Rooijen R, Aarts MGM, Harbinson J (2015) Natural genetic variation for acclimation of photosynthetic light use efficiency to growth irradiance in *Arabidopsis thaliana*. Plant Physiol 167: 1412–1429
Walters RG (2005) Towards an understanding of photosynthetic acclimation. J Exp Bot 56: 435–447
Watling JR, Ball MC, Woodrow IE (1997) The utilization of lightflecks for growth in four Australian rain-forest species. Funct Ecol 11: 231–239
Wayne PM, Bazzaz FA (1993) Birch seedling responses to daily time courses of light in experimental forest gaps and shadehouses. Ecology 74: 1500–1515
Yin ZH, Johnson GN (2000) Photosynthetic acclimation of higher plants to growth in fluctuating light environments. Photosynth Res 63: 97–107
Zheng Y, Blom T, Dixon M (2006) Moving lamps increase leaf photosynthetic capacity but not the growth of potted gerbera. Sci Hortic 107: 380–385

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Figures S1–S7 and Table S1.

Edited by A. Krieger-Liszkay