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Blue radiation stimulates photosynthetic induction in *Fagus sylvatica* L.

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Abstract

This study was designed to test the hypothesis that the spectral composition of incident radiation, as defined by the relative proportions of blue (B; λ_{max} = 455 nm) and red (R; λ_{max} = 625 nm) photons, can affect photosynthetic induction, since B photons stimulate stomatal opening and are more effectively absorbed by leaves than R photons. Different stages of photosynthetic induction, primarily determined by the photo-modulation of Rubisco activity and stomata opening, were investigated in dark-adapted leaves of *Fagus sylvatica* transferred to saturating irradiance [800µmol(photon) m^{-2} s^{-1}] at B/R ratios of 1/3, 1/1, or 3/1.

In agreement with our hypothesis, photosynthesis was induced faster by irradiance with a high B/R ratio (3/1); as demontrated by a higher IS_{60} (induction state 60 s after leaf illumination) and lower T_{90} (the time period required to reach 90 % of maximum steady-state photosynthesis). However, there were no differences in induction between leaves receiving equal (1/1) and low (1/3) B/R ratios. Electron transport was highly sensitive to radiation quality, exhibiting faster induction kinetics with increasing B/R ratio. Such stimulation of carbon-assimilatory processes corresponds with faster activation of Rubisco and lower non-photochemical quenching (NPQ) as the proportion of B photons is increased. In contrast, the kinetics of stomatal opening was independent of the spectral composition of incoming radiation. Since slightly higher absorption efficiency of high B/R radiation does not fully explain the changes in induction kinetics, the other possible mechanisms contributing to the stimulation of electron transport and Rubisco activity are discussed.

Additional keywords: blue/red ratio, electron transport, non-photochemical quenching, radiation quality, Rubisco activation, transient limitations of photosynthesis.

Introduction

Plants in natural environments are exposed to incident radiation of varying photon flux density (PFD) and spectral quality (Frankland 1986, Grant 1997, Murchie and Horton 1997), over a wide range of temporal and spatial scales (Campbell and Norman 1998, Navrátil *et al.* 2007). Above the canopy, the blue region of the solar spectrum is over-represented under cloudy-sky conditions compared with clear-sky conditions (Grant 1997),

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Abbreviations: *Ab* (*Rf*, *Tr*) – absorptance (reflectance, transmittance); B (R, FR) – blue (red, far-red) radiation; C_i – intercellular CO_2 concentration; Chl (Car) – chlorophyll (carotenoid); Chl-F – chlorophyll fluorescence; ETR – electron transport rate; g_s – stomatal conductance to water vapor; H (E, L) – high (equal, low) proportion of blue to red radiation; IS_{60} – induction state 60 s after leaf illumination; LS_{max} – maximum value of transient stomatal limitation; NPQ – non-photochemical quenching; P_N (P_N^*) – CO_2 assimilation rate (*without stomatal limitation); PFD – photon flux density; R_D – dark respiration rate; T_{90} – time required to reach 90 % of maximum steady-state; T_{S0} (T_{B0}) – time required to remove transient biochemical (stomatal) limitations; V_{Cmax} – maximum carboxylation rate; τ – time constant for Rubisco activation.
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particularly at low (10–20°) solar elevation angles (Navrátil *et al.* 2007, Urban *et al.* 2007a). Thus, the ratio between the blue (B; 400–500 nm) and red (R; 600–700 nm) spectral regions (B/R) varies from ≈ 0.5 (direct radiation) to ≈ 0.95 (diffuse solar radiation).

When solar radiation passes through a canopy, green and far red (FR) wavelengths are transmitted or reflected, while R and B wavelengths are preferentially absorbed (Grant 1997, Combes *et al.* 2000). Although absorptance of photons depends among others on pigment composition of pigment-protein-complexes, leaf absorptance of B photons is generally higher than that of R photons. Therefore, transmitted diffuse radiation in the understory is characterized by reduced R/FR (≈ 0.15) and B/R (≈ 0.35) ratios compared with that above the canopy (Frankland 1986, Campbell and Norman 1998). However, Navrátil *et al.* (2007) reported that direct solar radiation penetrating into the lower parts of coniferous canopies may have an increased B/R ratio up to 2.5, especially at low solar elevation angles (10–20°).

Photosynthesis is primarily reliant on the absorption of radiant energy by photosynthetic pigments, *i.e.* chlorophylls (Chls) and carotenoids (Cars). The absorption bands of Chls and Cars overlap in the B spectral region, while only Chls absorb in the R region (reviewed in Frankland 1986, Nobel 2005). Therefore, incident radiation should be absorbed more effectively as its B/R ratio increases (Grant 1997, Combes *et al.* 2000, Nobel 2005).

Blue radiation may control the activity of several enzymes (Thomas 1981), elicit the migration of chloroplasts in response to changes in PFD and solar tracking by leaves of certain plant species (Briggs and Christie 2002), and stimulate stomatal opening (Johnsson *et al.* 1976, Sharkey and Raschke 1981, Zeiger and Field 1982, Zeiger 1986, Kinoshita *et al.* 2001). B radiation causes wider opening of the stomatal aperture than R radiation at high PFDs. At low PFDs [below 15 μmol(photon) m^{-2} s^{-1}], stomata only open in response to B, while R radiation has no effect (Zeiger *et al.* 1981, Zeiger 1986, Kinoshita *et al.* 2001). B-photon-stimulated stomatal opening allows increased stomatal conductance of CO_2 through diffusion, followed by increases of CO_2 concentrations at both intercellular and chloroplast levels, consequently leading to greater uptake of CO_2. This probably contributes to the higher steady-state photosynthetic rates per unit leaf area under enhanced B/R radiation ratios (Zeiger *et al.* 1981, Matsuda *et al.* 2004).

Previous studies of photosynthetic induction in response to a sudden increase in PFD have focused on the temporal variability of the light environment (Pearcy 1990, Sassenrath-Cole and Pearcy 1992, Pearcy *et al.* 1994, Valladares *et al.* 1997, Allen and Pearcy 2000, Naumburg and Ellsworth 2000, Schulte *et al.* 2003, Urban *et al.* 2007b, Montgomery and Givnish 2008, Urban *et al.* 2008). However, effects of the spectral composition of incident PFD on photosynthetic induction have received only limited attention. To date, Johnsson *et al.* (1976) reported similar rates of opening for stomatal apertures of non-graminaceous leaves receiving B or R radiation; whereas, B radiation significantly stimulated the kinetics of stomatal response in graminaceous species. More recently, Tinoco-Ojanguren and Pearcy (1995) provided evidence that PFD quality, defined by R/FR ratio, had no significant effect on the dynamic photosynthetic characteristics of three tropical tree species.

Here we investigated the influence of incident radiation quality, defined by B/R ratio, on the rate of photosynthetic induction. B/R ratio was adjusted to between 3 and 0.33, covering the maximum range of its variability under natural light environments. Based on the above-mentioned physiological effects of B and R photons, we tested the hypothesis that an increase in the proportion of B in incident PFD will lead to faster photosynthetic induction and higher steady-state photosynthetic activity. Since the photosynthetic induction response is primarily determined by photo-modulation of Rubisco activity (Woodrow and Mott 1989, Hammond *et al.* 1998) and the photo-mediated opening of stomata (Valladares *et al.* 1997, Allen and Pearcy 2000), the responses of these two mechanisms limiting C assimilation were examined in sun leaves of European beech (*Fagus sylvatica* L.) seedlings.

Materials and methods

Plants: The study was conducted on five-year-old seedlings of European beech (*Fagus sylvatica* L.). A set of nine potted (local soil: ferric-podzols, loamy/sand-loamy, 30–40 % of gravel; 0.15 m^3 volume) seedlings was grown in the shade of old mature trees [daily photosynthetic PFD maximum 500 μmol(photon) m^{-2} s^{-1}] in a park at the Institute of Systems Biology and Ecology in Brno, SE of the Czech Republic (49°11′ N, 16°39′ E, altitude 250 m). The seedlings were exposed to natural environmental conditions and regularly irrigated. Physiological measurements were performed during July and August 2007, on fully-developed sun leaves. The third leaf from the top of each plant was measured and total chlorophyll (Chl) content *ca.* 0.20–0.24 g m^{-2} was estimated using a pre-calibrated Chl meter *SPAD-502 (Konica Minolta*, Osaka, Japan). The seedlings and their leaves were healthy without any signs of drought stress or photobleaching.

During the morning, seedlings were transferred from outdoor conditions to the air-conditioned laboratory (air temperature 20 °C) prior to physiological measurements. The plants were darkened for 45 minutes to ensure a comparable starting point, in particular full deactivation of Rubisco enzyme.

Estimation of leaf optical properties: Hemispherical spectral reflectance (*Rf*) and transmittance (*Tr*) of leaves in the 370–1000 nm spectral region was obtained using a portable spectroradiometer *Li-1800* (*LI-COR*, Lincoln, NE, USA) equipped with an integrating sphere *1800-12* (*LI-COR*, Lincoln, NE, USA). Hemispherical reflectance and transmittance of leaf samples were computed as:

$$Rf = I_{sr} / I_r, \text{ and} \tag{1}$$

$$Tr = I_{st} / I_r, \tag{2}$$

where I_{sr} and I_{st} are the fluxes of radiation measured from the adaxial leaf side. The reference scan (I_r) was taken with adaxial surface facing the sphere space for *Rf* and with abaxial surface facing the sphere space for *Tr* estimation, respectively. The efficiency of light absorption (absorptance; *Ab*) was calculated as $Ab = 1 - (Rf + Tr)$ (Navrátil *et al.* 2007).

Gas-exchange and fluorescence measurements: Simultaneous measurements of Chl *a* fluorescence (Chl-F) and H_2O/CO_2 fluxes were made using the commercial kinetic imaging fluorometer *FluorCam* (*Photon Systems Instruments*, Brno, Czech Republic) directly mounted on the sensor head of gas-exchange system *Li-6400* (*LI-COR*, Lincoln, NE, USA).

Chl-F induction kinetics (Kautsky effect) of pre-darkened leaves was measured in the red fluorescence band (695–750 nm). The lens of the fluorometer was held parallel to the adaxial leaf surface at ca. 7 cm distance. The fluorometer uses a CCD camera to capture Chl-F images (12-bit resolution in 512 × 512 pixels) as a function of time under different irradiances. Low-intensity measuring-pulses (10 μs pulses of an intensity of ca. 0.003 μmol(photon) m^{-2} s^{-1}), generated by blue LEDs (*LXHL-PR09, Philips Lumileds Lighting Company*, San Jose, USA), were applied to detect the initial Chl-F (F_O) in dark-adapted leaves and actual Chl-F (F_S) in light-adapted leaves. Saturating-pulses [0.8 s of *ca.* 6000 μmol(photon) m^{-2} s^{-1} intensity], generated by red (*LXHL-PH09*) and blue LEDs (*LXHL-PR09*, *Philips Lumileds Lighting Company*, San Jose, USA), were used to detect the maximum Chl-F in dark (F_M) and in light-adapted (F_M') plant states, respectively.

The dark respiration rate (R_D) and maximum quantum yield of photosystem (PS) II photochemistry, defined as $F_V/F_M = (F_M–F_O)/F_M$, were measured at the end of the dark period. Subsequently, the middle part (6 cm^2) of adaxial leaf side was exposed to continuous saturating radiation [PFD; 800 μmol(photon) m^{-2} s^{-1}] using the blue (λ_{max} = 455 nm, spectral half-width 25 nm) and red (λ_{max} = 625 nm, spectral half-width 25 nm) LEDs (*Philips Lumileds Lighting Company*, San Jose, USA), which are a part of *FluorCam* (*PSI*, Brno, Czech Republic). The following combinations of blue and red PFDs were used: 600+200, 400+400, and 200+600 μmol(photon) m^{-2} s^{-1}. The above mentioned irradiances of B and R LED sources were set up separately and checked using *Quantum Sensor LI-190SA* (*LI-COR*, Lincoln, NE, USA). Combination of those irradiances provided the high (3/1, H), equal (1/1, E) or low (1/3, L) proportions of blue to red PFDs. The other microclimatic conditions inside the assimilation chamber were kept constant throughout the measurements; *i.e.*, ambient CO_2 concentration [375 ± 5 μmol(CO_2) mol^{-1}], air humidity (60 ± 5 %), and leaf temperature (20 °C). The air flow rate through the assimilation chamber was maintained at 500 μmol(air) s^{-1}.

The time course of actual photosynthetic characteristics, *i.e.*, CO_2 assimilation rate (P_N), stomatal conductance to water vapor (g_s), and intercellular CO_2 concentration (C_i), were automatically recorded (at 10 s intervals) using a *Li-6400* (*LI-COR*, USA) until maximum P_N and maximum g_s were stable (approximately 1 h).

Actual Chl-F was detected every 30 s. In addition, saturation pulses were applied 1, 2.5, 4, 6, 8, 10, 15, 20, 30, 40, and 50 min after illumination of the leaf by saturating PFD, to evaluate the electron transport rate (ETR) and non-photochemical quenching (NPQ) (Genty *et al.* 1989):

$$\mathrm{ETR} = \frac{(\mathrm{F}_M' - \mathrm{F}_S)}{\mathrm{F}_M'}\ 0.5\ (\mathrm{PFD}_{blue}\ \mathrm{Ab}^{blue} + \mathrm{PFD}_{red}\ \mathrm{Ab}^{red}) \tag{3}$$

$$\mathrm{NPQ} = \frac{\mathrm{F}_M - \mathrm{F}_M'}{\mathrm{F}_M'} \tag{4}$$

where the factor 0.5 assumes that the incident quanta, used to excite both PSII and PSI, are equal, *Ab* is the absorptance of the leaves determined at 455 nm (blue) and 625 nm (red), *i.e.* 0.899 and 0.793 (Fig. 1), and PFD is the actual flux density of blue and red photons.

After complete photosynthetic induction (*ca.* 60 min), the initial part of an P_N/C_i response curve was produced starting at an ambient CO_2 concentration of 375 μmol(CO_2) mol^{-1} in the leaf chamber and decreasing in steps until 50 μmol(CO_2) mol^{-1} was reached. Values from the P_N/C_i response curves were used to calculate the maximum carboxylation rate (V_{Cmax}) and CO_2 compensation concentration in the absence of photorespiration (Γ^*) using the equations of Farquhar *et al.* (1980) and Michaelis-Menten constants of Rubisco carboxylation and oxygenation at 20°C, *i.e.* 160 μmol(CO_2) mol^{-1} and 380 mmol(O_2) mol^{-1} (Harley *et al.* 1992).

Modelling induction responses: The induction responses of P_N and g_s were modelled according the algorithm described in Zipperlen and Press (1997). A sigmoidal function (Eq. 5) was fitted to the induction response curves and the residual sum of squares was minimized using:

$$x = \frac{\min - \max}{1 + \left(\frac{t}{i}\right)^s} + \max \tag{5}$$

where x is the actual P_N or g_s at time *t*; min is the asymptotic minimum P_N or g_s before leaf illumination; max is the asymptotic maximum P_N or g_s attained during the saturating light period (P_{Nmax}, g_{smax}); t is time; i is the time of inflection; and s is a slope parameter. Estimations of the time required to reach 90 % of full P_N and g_s induction [$T_{90}(P_N)$, $T_{90}(g_s)$] were obtained by solving Eq. 5 for time (Eq. 6) and substituting in values of 90 % P_{Nmax} and g_{smax},

$$T_{90} = i\left(\frac{0.9\max - \min}{0.1\max}\right)^{1/s} \quad (6)$$

The induction response of ETR was modelled using an exponential function (Schulte *et al.* 2003):

$$\mathrm{ETR} = \mathrm{ETR}_{max} - \mathrm{ETR}_{max}\,\mathrm{e}^{-bt} \quad (7)$$

where ETR is the actual rate of electron transport at time t. The exponential coefficient b was estimated by Newton's least-square procedure. The time required to reach 90 % of ETR_{max} was calculated as:

$$T_{90}(\mathrm{ETR}) = \ln(0.1)\cdot\left(-\frac{1}{b}\right) \quad (8)$$

The induction states of P_N and ETR at 60 s after leaf illumination were calculated following Naumburg and Ellsworth (2000):

$$\mathrm{IS}_{60}(x) = \frac{x - \min}{\max - \min}\cdot 100 \quad (9)$$

where x is the transient P_N or ETR 60s after leaf illumination; min is the steady-state minimum of P_N (*i.e.*, dark respiration rate) or ETR (*i.e.*, 0 μmol(e$^-$) m^{-2} s^{-1}) before leaf illumination; max is the steady-state maximum of P_N or ETR attained during the saturating light period (P_{Nmax} or ETR_{max}).

Model of induction limitations: The model proposed by Woodrow and Mott (1989) was used to calculate transient non-stomatal (usually referred as transient biochemical limitation; *e.g.* Hammond *et al.* 1998, Schulte *et al.* 2003) and stomatal limitations that disappear during photo synthetic induction. In this model, stomatal limitations to photosynthesis are removed by recalculating the photosynthetic rate to a constant intercellular CO_2 concentration (C_i). The rate of CO_2 assimilation without stomatal limitation (P_N^*) was calculated as

$$P_N^* = \frac{(P_N + R_D)(C_{if} - \Gamma^*)}{C_i - \Gamma^*} - R_D \quad (10)$$

where P_N is the actual CO_2 assimilation rate, C_{if} is the final C_i (C_i at the end of the induction period), Γ^* is the CO_2 compensation concentration in the absence of photorespiration, and R_D is the dark respiration rate. Hence,

$$\mathrm{LS} = \frac{(P_N^* - P_N)}{(P_{Nmax} + R_D)} \quad (11)$$

represents those transient stomatal limitations (LS) that disappear during the photosynthetic induction phase. P_{Nmax} is the maximum CO_2 assimilation rate at the end of the induction period. The time constant for Rubisco activation (τ) was determined from a plot of the natural logarithm of P_{Nmax} minus the measured assimilation rate, [$\ln(P_{Nmax} - P_N)$], over time. The slow phase of photosynthetic activation (*i.e.* from two minutes after leaf illumination onwards) corresponds to the linear portion of this plot, where the rate constant represents the absolute value of the slope (Hammond *et al.* 1998). The general equation describing the linear portion of the log plot is:

$$P_N = P_{Nmax} - (P_{Nmax} - P_{Ni})\mathrm{e}^{-\tau t} \quad (12)$$

where t is time and the subscript i denotes the extrapolated initial assimilation rate at time zero (*i.e.* the rate of dark respiration R_D here).

Statistical analysis: One-way and two-way *ANOVA* (*Scheffe* test) were applied to detect the statistically significant differences ($p<0.05$) in photosynthetic steady-state and induction parameters among leaves exposed to different spectral compositions of PFD. All statistical tests were performed with *STATISTICA ver.7.0* (*StatSoft Inc*., Tulsa, USA).

Results

Steady-state photosynthetic characteristics: Fig. 1 shows the typical spectral optical properties of a mature beech leaf, measured from the adaxial leaf side, with high absorption of incident radiation in the B part of the spectrum caused by Chls and Cars. According to our measurements, mean absorptance (± standard deviation) in the B part of the spectrum (400–500 nm) was 0.899 ± 0.015 and in the R part (600–700 nm) it was 0.793 ± 0.058.

In accordance with our initial hypothesis, slight stimulation of the light-saturated stomatal conductance (g_{smax}) occurred as the proportion of B radiation was increased (Table 1). Leaves exposed to PFD with a high (H) B/R had considerably higher g_{smax} (by 15.5 %) than leaves at a low (L) B/R ratio. Higher g_{smax} at H B/R subsequently led to higher intercellular CO_2 concentrations (C_{if}) than those at equal (E) B/R (by 7.5 %) or L B/R ratio (by 12 %). However, there was no effect of spectral composition on CO_2 assimilation rate (P_{Nmax}) under saturating PFD (Table 1). In contrast, light-saturated ETR (ETR_{max}) was stimulated by B radiation. Maximum rate of Rubisco carboxylation (V_{Cmax}) was higher by 20 % under L B/R radiation compared with E and H B/R radiation. Likewise, PFD with H B/R led to a

Table 1. Summary of photosynthetic parameters (mean ± SD) estimated under steady-state conditions in leaves of European beech (*Fagus sylvatica*) exposed to saturating photon flux density [PFD; 800 μmol(photon) m^{-2} s^{-1}] of differing spectral composition. The proportion of blue to red light intensities was set to high (3/1, H), equal (1/1, E) or low (1/3, L). Parameters were estimated at the end of the photosynthetic induction: P_{Nmax} – PFD saturated rate of CO_2 assimilation; g_{smax} – PFD saturated stomatal conductance; C_{if} – intercellular CO_2 concentration; V_{Cmax} – PFD saturated rate of carboxylation, ETR_{max} – PFD saturated rate of electron transport rate. ETR_{max} represents mean value calculated from all individual leaf pixels (260 000). Identical letters indicate homogeneous groups with statistically non-significant differences (p>0.05); n = 9.

	P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	g_{smax} [mol(H_2O) m^{-2} s^{-1}]	C_{if} [μmol(CO_2) mol^{-1}]	V_{Cmax} [μmol(CO_2) m^{-2} s^{-1}]	ETR_{max} [μmol(e^-) m^{-2} s^{-1}]
H	9.4±0.6^a	0.15±0.01^a	263±9^a	25±2.3^a	92±2.8^a
E	9.3±0.5^a	0.14±0.01^{a,b}	245±9^b	26±2.4^a	84±3.9^b
L	9.8±0.5^a	0.13±0.01^b	235±9^b	31±2.9^b	70±4.5^c

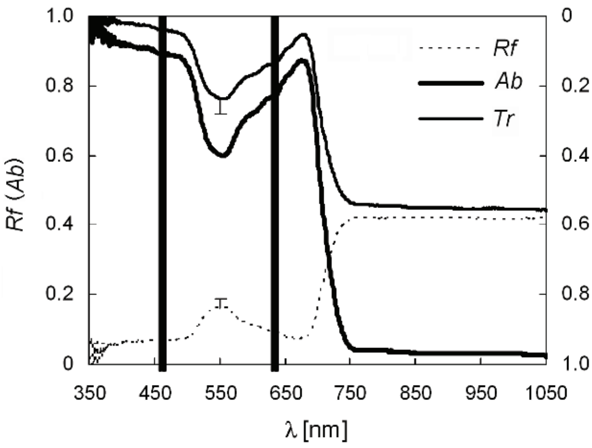

Fig. 1. Relationship between hemispherical reflectance (*Rf*), transmittance (*Tr*) and absorptance [*Ab*; calculated as 1 – (*Rf* + *Tr*)] and the wavelength of incident radiation in studied leaves of European beech (*Fagus sylvatica*). Means ± SD (*bars*) are presented (n = 9). Vertical lines indicate maximum of LED emissions (*see* Materials and methods).

10 % increase in ETR_{max} compared with E B/R, and a 31 % increase compared with L B/R (Table 1). The rates of dark respiration, initial stomatal conductance and F_V/F_M before leaf illumination ranged within the intervals 0.75–1.0 μmol(CO_2) m^{-2} s^{-1}, 0.01–0.02 mol(H_2O) m^{-2} s^{-1} and 0.80–0.82, respectively (data not shown).

Photosynthetic induction characteristics: Faster photosynthetic induction occurred, as demonstrated by higher $IS_{60}(P_N)$ and shorter $T_{90}(P_N)$ values (p<0.05), in leaves exposed to irradiance with a H proportion of B photons, whereas leaves illuminated by irradiances with both E B/R and L B/R ratios exhibited slower photosynthetic induction (Fig. 2). There were no statistically significant differences (p>0.05) between the E and L variants. The development of the induction state of P_N during the whole process of photosynthetic induction (Fig. 3) was promoted in the H variant compared with other variants. Moreover, the rate of induction during the middle phase of P_N induction (the time interval from 300 to 600 s) appears to be quicker in variant E as compared to L. Thus, although differences between E and L illuminated beech leaves are not revealed by the basic P_N-induction parameters, the time-course assessment (Fig. 3) shows that E illumination exerts transient stimulation of photosynthetic induction that disappears during the slow induction phase.

Induction parameters of electron transport (Fig. 2), derived from ETR induction curves (Fig. 4), exhibited high sensitivity to the spectral composition of incoming

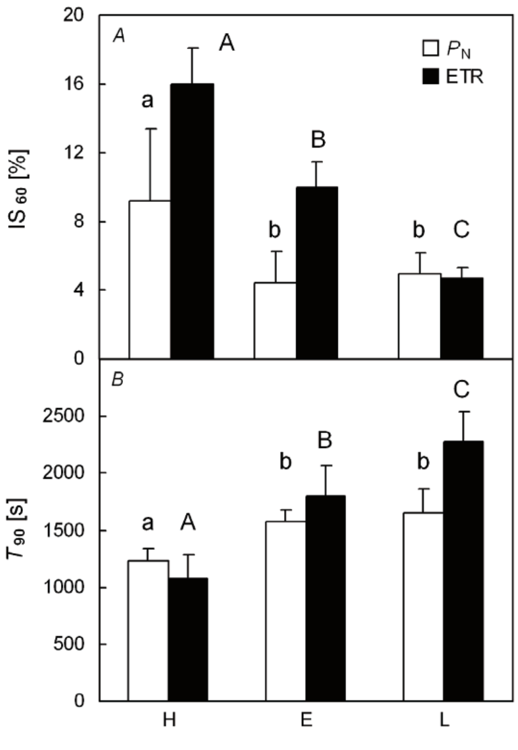

Fig. 2. Analysis of induction states after a 60-s exposure to saturating irradiance (IS_{60}; *A*), and time required to reach 90 % (T_{90}; *B*) of the maximum light-saturated rate of CO_2 assimilation (P_N; *open bars*) and electron transport (ETR; *filled bars*). Values are means ± SD (n = 9) for leaves of *Fagus sylvatica* exposed to photon flux density with high (H; 3/1), equal (E; 1/1) and low (L; 1/3) blue to red ratio. Identical letters indicate homogeneous groups with statistically non-significant differences (p>0.05).

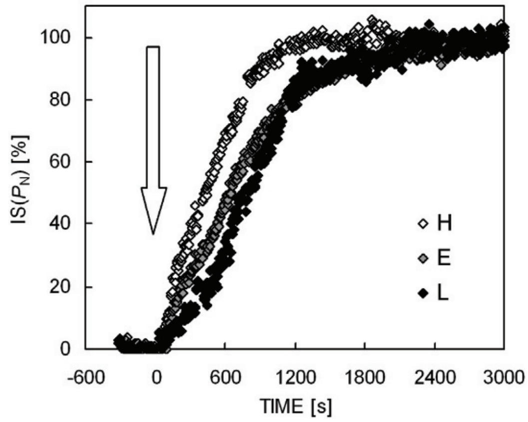

Fig. 3. Representative time courses of the actual induction state of CO_2 assimilation rate [$IS(P_N)$] in leaves of *Fagus sylvatica* exposed to saturating photon flux density [800 μmol(photon) m^{-2} s^{-1}] with high (H; 3/1), equal (E; 1/1) and low (L; 1/3) blue to red light ratio. Time course of $IS(P_N)$ was calculated according to Eq. 7, $IS(A) = [(P_N–R_D)/(P_{Nmax}–R_D)]*100$, where P_N is the actual rate of CO_2 assimilation, P_{Nmax} is the light saturated rate of CO_2 assimilation at the end of the photosynthetic induction period, and R_D is the rate of dark respiration before leaf illumination. The arrow indicates the instantaneous change from darkness to continuous saturating photon flux density.

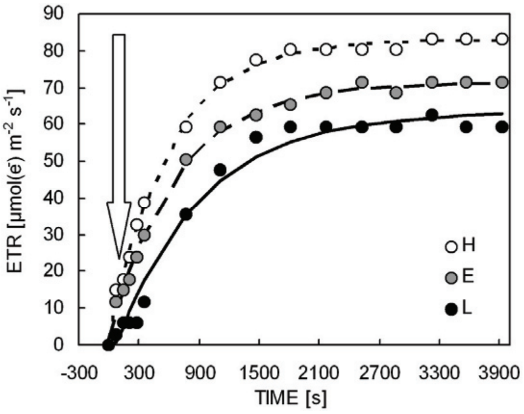

Fig. 4. Representative time courses of electron transport rate (ETR) during photosynthetic induction in leaves of *Fagus sylvatica* exposed to saturating photon flux density [800 μmol(photon) m^{-2} s^{-1}] with high (H; 3/1), equal (E; 1/1) and low (L; 1/3) blue to red light ratio. The data were fitted using an exponential model (Eq. 8); $r^2 = 0.87–0.95$. The arrow indicates the instantaneous change from darkness to continuous saturating photon flux density.

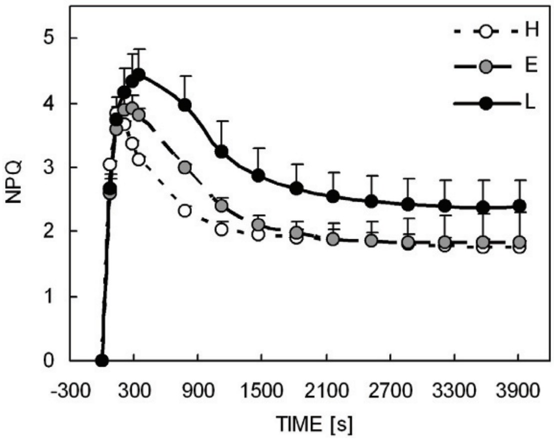

Fig. 5. Time courses of non-photochemical quenching (NPQ) during photosynthetic induction in leaves of *Fagus sylvatica* exposed to saturating photon flux density [800 μmol(photon) m^{-2} s^{-1}] with high (H; 3/1), equal (E; 1/1) and low (L; 1/3) blue to red light ratio. Mean values (*circles*) and SD (*bars*) are presented; $n = 9$.

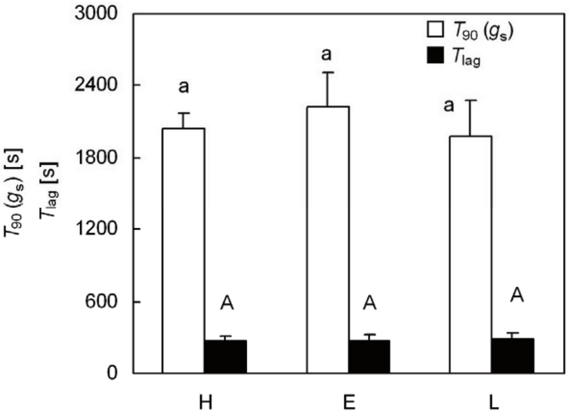

Fig. 6. Analysis of time required to reach 90 % of light saturated stomatal conductance ($T_{90}(g_s)$; *open bars*) and time-lag for stomatal opening (T_{lag}; *filled bars*). Values are means ± SD ($n = 9$) for leaves of *Fagus sylvatica* exposed to photon flux density with high (H; 3/1), equal (E; 1/1) and low (L; 1/3) blue to red light ratio. Identical letters indicate homogeneous groups with statistically non-significant differences ($p>0.05$). The time-lag for stomatal opening was calculated according the algorithm described in Naumburg and Ellsworth (2000).

radiation. The stimulatory effect of B photons, demonstrated by an increase in IS_{60} and decrease in T_{90} parameters, was more pronounced for the induction of ETR than for the induction of P_N (Fig. 2). For example, in variant H IS_{60}(ETR) was 210 % higher than variant L; while, the corresponding increase in $IS_{60}(P_N)$ was only 80 %. Upon illumination by H and E B/R the transient NPQ maxima were reduced by 13 % compared to L B/R (Fig. 5). Increasing the proportion of B photons also led to an earlier increase in NPQ, which reached maximum values from 350 second for L B/R compared with 230 second for H R/B, and significantly accelerated the subsequent decline and plateau in NPQ (Fig. 5).

Surprisingly, there was no evidence that PFD spectral composition affected stomatal induction, *i.e.* the time required to reach 90 % of light-saturated g_{smax} [$T_{90}(g_s)$] and the time-lag for stomatal opening (Fig. 6). $T_{90}(g_s)$ was within the interval 1980–2220 seconds and the mean time-lag before stomata started to open was 275 seconds irrespective of radiation type.

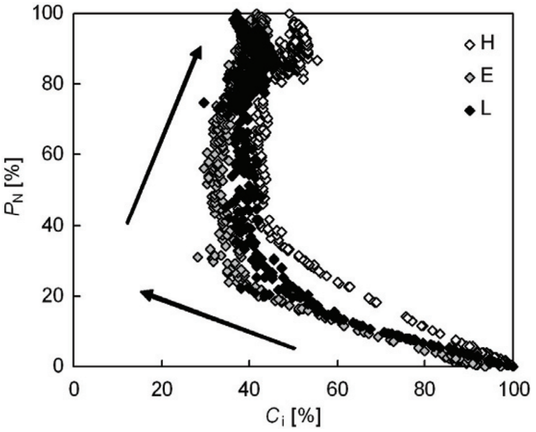

Fig. 7. Typical examples of relationship between CO_2 assimilation rate (P_N) and intercellular CO_2 concentration (C_i) during photosynthetic induction in *Fagus sylvatica* leaves exposed to saturating photon flux density [800 μmol(photon) m^{-2} s^{-1}] with high (H; 3/1), equal (E; 1/1) and low (L; 1/3) blue to red ratio. The P_N values were normalized to light-saturated steady-state CO_2 assimilation rate (P_{Nmax}), and C_i values were normalized to intercellular CO_2 concentration in the dark before the leaf illumination. The arrows indicate the development of the relationship after leaf illumination.

Transient limitations during photosynthetic induction: Transient non-stomatal (biochemical) and stomatal limitations were analyzed by comparing P_N and C_i during photosynthetic induction (Fig. 7). Although normalized P_N/C_i curves are presented here, the initial C_i before leaf illumination ranged within a narrow interval from 450 to 480 μmol(CO_2) mol^{-1} in all leaves studied and different B/R ratios had no effect on Γ^* that ranged between 50 and 80 μmol(CO_2) mol^{-1}. In general, the initial part of the P_N/C_i curve is predominately limited by transient biochemical limitations, while the second part is limited more by transient stomatal limitations which disappear during photosynthetic induction.

Only leaves exposed to PFD with a H B/R exhibited lower maximum values of transient stomatal limitation (LS_{max}; by 25–28 %; Table 2) as well as a shorter period of time before transient stomatal limitations passed (T_{S0}; by 32–38 %; Fig. 8), not leaves illuminated with E B/R and L B/R ratios. After accounting for stomatal limitation, the remaining induction limitation was primarily the result of transient non-stomatal limitation, in particular Rubisco activation. Generally, transient non-stomatal limitations appeared more sensitive to the spectral composition of incident PFD than transient stomatal limitations. In particular, the time constant of Rubisco activation (τ), reflecting the rate of Rubisco activation, was very sensitive to the spectral composition of incident radiation. The proportional increase in B radiation, from L to E and H B/R ratio, led to a significant decrease in τ by 18 % and 68 % (Table 2), respectively, and consequently transient biochemical limitations (T_{B0}) were removed sooner (16 % and 27 %) (Fig. 8). All these differences are statistically significant ($p<0.05$).

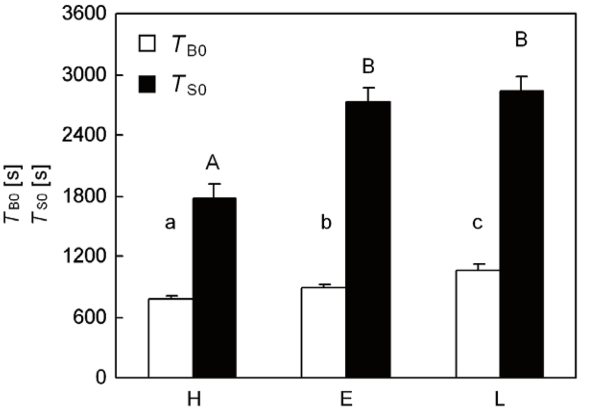

Fig. 8. Analysis of the times required to remove transient biochemical (T_{B0}; *open bars*) and stomatal limitations (T_{S0}; *filled bars*) during photosynthetic induction. Values are means ± SD ($n = 9$) for leaves of *Fagus sylvatica* exposed to photon flux density with high (H; 3/1), equal (E; 1/1) and low (L; 1/3) blue to red light ratio. Identical letters indicate homogeneous groups with statistically non-significant differences ($p>0.05$).

Table 2. The time constant for Rubisco activation (τ) and maximum transient stomatal limitations (LS_{max}) analysed during photosynthetic induction. Values are means ± standard deviations ($n = 9$) for leaves of *Fagus sylvatica* exposed to photon flux density with high (H; 3/1), equal (E; 1/1) and low (L; 1/3) blue to red light ratio. Identical letters indicate homogeneous groups with statistically non-significant differences ($p>0.05$).

	τ [s]	LS_{max} [%]
H	540±190^a	20.3±6.2^a
E	1380±260^b	28.0±2.1^b
L	1680±246^c	28.1±6.6^b

Discussion

Our hypothesis that leaves exposed to PFD with a higher proportion of B photons would give higher steady-state values of P_{Nmax} and g_{smax}, was not supported by the results, but there was faster induction kinetics under H B/R ratio than in leaves exposed to the same PFD with a higher proportion of R photons. Of the two underlying mechanisms that we proposed (*1*) B photons did not stimulate the opening of stomatal aperture as expected but (*2*) B photons were absorbed more effectively by leaves than R photons, so may have been responsible for stimulation of the ETR.

Steady-state responses: Surprisingly, there was apparently no stimulation of steady-state values of P_{Nmax}

in *F. sylvatica* leaves by B radiation at saturating PFD. Nevertheless, this result is consistent with earlier findings that P_{Nmax} of several broadleaf and coniferous species is unresponsive to imposed changes in the spectral composition of incident radiation (Clark and Lister 1975). In general, blue light is depleted faster across the transverse leaf profile than red light. Cui *et al.* (1991) demonstrated that up to 95 % of both blue and red light was attenuated by the initial 150 μm of palisade tissue in spinach sun leaves. However, subsequent visualizing of Chl fluorescence profiles demonstrated that light is absorbed at greater depths than predicted from fibre-optic measurements, with 50 % of blue light reaching 125 μm depth (Vogelmann and Evans 2002).

In contrast to P_{Nmax}, $\mathrm{ETR}_{\mathrm{max}}$ was highly stimulated by B radiation (Table 1). Derivation of ETR from the actual PSII photochemical efficiency considered the different leaf absorptances of the H, E and L B/R ratios (Fig. 1), however, an equal distribution of absorbed photons between PSII and PSI was assumed (Eq. 3). Although this assumption is reasonable under many experimental conditions, it can not be assumed if changes occur in the spectral composition of incident light or absorption cross sections of PSII and PSI (Baker *et al.* 2007). Absorption spectra of isolated photosystems (Melis *et al.* 1987) and low temperature excitation spectrum of Chl *a* fluorescence measured on intact leaves indicated that different spectral compositions of H, E and L B/R radiation ratios should not significantly alter the portion of absorbed photons reaching PSII. Thus, although an equal distribution of absorbed light between PSII and PSI (and inferential absolute ETR values) cannot be confirmed for the illuminations used in presented study, the increase in ETR that occurred as the proportion of B radiation was increased does support this assertion. Although, some contribution of the water-water cycle and/or cyclic electron transport to enhance the steady state ETR cannot be excluded (Genty *et al.* 1989), increased Rubisco activity, particularly in favor of oxygenation, seems to be the main process that restores the balance between the enhanced production of ATP and NADPH and their consumption (Igamberdiev *et al.* 2001).

In addition, we report only slight stimulation of steady-state g_{smax} by higher proportions of B photons in the incident radiation (Table 1). Based on published data we suggest that the stimulating effect of B radiation on stomatal response occurs more frequently in graminaceous species (Johnsson *et al.* 1976) and under low PFDs (Zeiger and Field 1982) than in herbaceous and woody species or at high PFDs. The latter authors, experimenting on *Malva parviflora*, concluded that B (457.9 nm) and R (640 nm) radiation had similar effects on conductance at intensities higher than 200 μmol(photon) $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$, a finding that fully agrees with our results.

Dynamic responses: Contrary to our initial presumption, B/R ratio had no effect on the induction kinetics of stomatal opening. Kana and Miller (1977), likewise, reported that opening rates of stomatal apertures were unaffected by saturating intensities of red, green, and blue radiation in *Vicia faba*. Stomatal responses may be species-specific, as demonstrated by Johnsson *et al.* (1976). In agreement with our results, they found that the kinetics of induced stomatal responses in four non-graminaceous species (*Ricinum communis*, *Vicia faba*, *Iris tectorum* and *Dipsacus silvester*) responded similarly to pulses of B and R radiation; however, they reported a stimulating effect of blue radiation compared to red radiation in another four graminaceous species (*Lolium perenne*, *Panicum milaceum*, *Trisetum flavescens* and *Arrhenatherum elatius*) when the stomatal response started immediately after the pulse of B radiation.

Although stomatal opening was not stimulated, the induction kinetics presented here (Figs. 3,4), and their induction parameters (Fig. 2), support the initial hypothesis that B photons partially stimulate induction of P_{N} and particularly stimulate the induction of ETR. Based on the absorption spectra of isolated photosystems (Melis *et al.* 1987), we consider that those changes in ETR induction kinetics that occur on illumination with different spectral compositions, reflect real effects on PSII photochemical de-excitation, rather than an anomaly due to a changing portion of absorbed photons reaching PSII.

The increase of the NPQ after leaf illumination (Fig. 5) reflects induction of ΔpH-dependent non-radiative dissipation of absorbed excitation energy within PSII (during the period when light absorption exceeds the capacity of PSII photochemistry), whereas its partial relaxation is connected to the activation of photosynthetic reactions (Baker *et al.* 2007). Thus, both the lower NPQ maximum and promotion of its relaxation upon illumination with E and H B/R radiation provide strong evidence that light enriched in B-photons directly stimulates the induction of processes enhancing PSII photochemical de-excitation capacity.

We suggest the following mechanism of B-photon-stimulated induction. More-efficient absorption of B than R photons by leaves results in the stimulation of ETR induction. Particularly during the first minute of illumination, cyclic electron transport around PSI markedly contributes to total electron transport capacity and considerably enhances ΔpH formation (Fan *et al.* 2007). Thus, stimulated ETR leads to faster ATP production (Farquhar *et al.* 1980, Foyer *et al.* 1990, Nobel 2005) and may facilitate activation of Rubisco activase (Maxwell *et al.* 1999, Portis *et al.* 2008). ETR can affect Rubisco activase activity *via* processes related to the actual ATP/ADP ratio in the stroma and trans-membrane ΔpH; however ΔpH does not seem to be directly involved in the modulation of Rubisco activase activity (Maxwell *et al.* 1999). Increased Rubisco activase activity may consequently result in faster Rubisco activation (Portis *et al.* 2008), demonstrated here by lower τ values (Table 2) at the higher B/R ratio, and corresponds with faster induction of carbon assimilatory processes with an increasing proportion of B photons.

Blue radiation is also known to cause changes in the activity of several enzymes *via* its absorption by flavins (flavin mononucleotide – FMN and flavin adenine – FAD) serving as co-enzymes. Examples from higher plants are glycolate oxidase and NAD-malate dehydrogenase, both of which are inhibited by B radiation in the presence of FMN, and nitrate reductase, which is activated by blue radiation in the presence of FAD (Thomas 1981). However, we are not aware of any earlier study documenting changes in Rubisco activity due to the spectral composition of photosynthetically active radiation.

From the measured leaf absorptances in the B and R spectral regions it is straightforward to determine the absorptances 0.819, 0.845 and 0.872 for L, E and H illumination, respectively. This increase of less than 7 % in photons absorbed by the H compared with the L variant can account neither for the concomitant 30 % ETR_{max} stimulation (Table 1) nor for the pronounced acceleration of ETR induction kinetics (Figs. 2,4). Thus, we hypothesize that an additional mechanism could contribute to the stimulation of both ETR induction and Rubisco activation under a high B/R ratio.

The absorption of B photons is inevitably associated with a substantial energy loss due to the non-radiative transition from the second-excited singlet of Chl *a* molecules to their first-excited state, preceding photochemical conversion in the reaction centers of both photosystems. Therefore, an increased B/R ratio can considerably contribute to the thermal dissipation of absorbed excitation energy and cause an enhanced thermo-optical effect (Cseh *et al.* 2000) that would significantly increase the local temperature of pigment-protein complexes (Dobrikova *et al.* 2003). We infer that the heat is dispersed over the thylakoid membrane and can lead to an increase in the ETR as diffusion of plastoquinone and plastocyanine is facilitated (Barber *et al.* 1984). As the ETR exhibits Q_{10} close to 2 over the temperature range 10–30 °C (Yamori *et al.* 2008), an increase in local temperature of 4.5 °C is needed to change the membrane fluidity to account for a 30 % difference in ETR between L and H B/R ratios.

Conclusion: The main findings of our work are that the spectral composition of radiation at saturating PFD did not affect stomatal opening but affected biochemical aspects of photosynthetic induction. Electron transport was highly sensitive to radiation quality, exhibiting faster induction kinetics with increasing B/R ratio. Thus, the enhanced production of ATP seems to be responsible for the faster Rubisco activation and consequent stimulation of photosynthetic induction as the proportion of B photons is increased.

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