

RESEARCH PAPER

What determines the complex kinetics of stomatal conductance under blueless PAR in *Festuca arundinacea*? Subsequent effects on leaf transpiration

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Abstract

Light quality and, in particular, its content of blue light is involved in plant functioning and morphogenesis. Blue light variation frequently occurs within a stand as shaded zones are characterized by a simultaneous decrease of PAR and blue light levels which both affect plant functioning, for example, gas exchange. However, little is known about the effects of low blue light itself on gas exchange. The aims of the present study were (i) to characterize stomatal behaviour in *Festuca arundinacea* leaves through leaf gas exchange measurements in response to a sudden reduction in blue light, and (ii) to test the putative role of C_i on blue light gas exchange responses. An infrared gas analyser (IRGA) was used with light transmission filters to study stomatal conductance (g_s), transpiration (T_r), assimilation (A), and intercellular concentration of CO_2 (C_i) responses to blueless PAR ($1.80 \mu\text{mol m}^{-2} \text{s}^{-1}$). The results were compared with those obtained under a neutral filter supplying a similar photosynthetic efficiency to the blueless PAR filter. It was shown that the reduction of blue light triggered a drastic and instantaneous decrease of g_s by 43.2% and of T_r by 40.0%, but a gradual stomatal reopening began 20 min after the start of the low blue light treatment, thus leading to new steady-states. This new stomatal equilibrium was supposed to be related to C_i . The results were confirmed in more developed plants although they exhibited delayed and less marked responses. It is concluded that stomatal responses to blue light could play a key role in photomorphogenetic mechanisms through their effect on transpiration.

Key words: Leaf growth, light, photomorphogenesis, photosynthesis, tall fescue.

Introduction

Light quality is considered to play a key role in plant architecture and the dynamics of vegetation (Kasperbauer and Hunt, 1992; Ballaré *et al.*, 1997), through wavelengths known as morphogenetically active radiation (MAR) (Varlet-Grancher *et al.*, 1993a). During plant development, the light phylloclimate (Chelle, 2005) changes as a result of (i) geometric interactions between incident light and phytoelements of the plant canopy and (ii) optical properties of vegetal stands. Geometric interactions lead to the formation

of shaded zones which occur both within plants and between plants. Light phylloclimate variations also result from the optical properties of the leaves (Smith, 1982), i.e. their photosynthetic pigments that mainly absorb in the blue and red wavelengths. Therefore, shaded zones within a plant canopy are also characterized by a decrease in (i) the red:far-red ratio (R:FR), (ii) the photosynthetically active radiation (PAR, 400–700 nm) including (iii) a decrease in a large part of the blue light (350–500 nm). As a result,

Abbreviations: A , rate of CO_2 assimilation; C_i , intercellular concentration of CO_2 ; g_s , stomatal conductance; MAR, morphogenetically active radiation; PAR, photosynthetically active radiation; PFD, photosynthetic flux density, ϕ_c , phytochromes photoequilibrium; ζ , red/far red ratio; T_r , transpiration rate; VPD, vapour pressure deficit.

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there are spatial, temporal, and directional light quality variations within a stand due to environmental factors such as the sun's course, cloudiness or the wind (Combes *et al.*, 2000; Escobar-Gutiérrez *et al.*, 2009). These variations in light quality (i.e. in the solar radiation spectrum) act as photomorphogenic signals sensed by plants through their photoreceptors. Variations in blue light level are perceived by two main photoreceptors: cryptochromes and phototropins (Lin, 2002) which are two systems acting as photon counters (Smith, 1982) in ultraviolet A (UVA) and blue light. Cryptochromes are active within the range of 390–530 nm with a fairly flat response between 390 nm and 480 nm (Ahmad *et al.*, 2002), whereas phototropin activity shows a clear peak at 450 nm (Christie *et al.*, 1998). In practice, UVA and blue light signals are usually characterized by the photon irradiance integrated over various wavebands within the 350–500 nm region (Varlet-Grancher *et al.*, 1993b). The perception of blue light through these photoreceptors allows plants to sense their nearby environment and, in particular, the intensity of competition for light. Consequently, blue light is known to trigger a large variety of photomorphogenic (*sensu lato*) responses in plants (Casal and Alvarez, 1988; Ballaré and Casal, 2000; Christie and Briggs, 2001) through a range of mechanisms at the molecular, cellular (Laseve *et al.*, 1999), and organ levels (Cosgrove and Green, 1981).

Besides its morphogenic effects, blue light also influences plant functioning. At the leaf scale, fluctuations in blue light involve changes in both energy balance components (Jones, 1992) and in gas exchange dynamics through stomatal functioning. Blue light effects on stomatal behaviour have been a topical issue for several decades (Zeiger *et al.*, 1987; Gautier, 1991; Shimazaki *et al.*, 2007; Lawson, 2009). For example, it has been well documented that blue light pulses

induced a transient stomatal opening in various species (Assmann, 1988), that may be important for the optimization of water use efficiency (Karlsson and Assmann, 1990). Nevertheless, the blue-light regime is embedded within a tangled network of interacting environmental factors that concomitantly affect stomatal functioning which, in turn, modifies these factors (Fig. 1). Briefly, the blue light effect on stomata could lead to variations of intercellular CO₂ concentration (*C_i*) and, consequently, of leaf assimilation rate which also depends on electron flow supplied by the PAR. Besides, leaf transpiration rate is both mediated by incident thermal radiation and by stomatal opening. Such mediation of leaf transpiration by blue light has been little studied (Brogardh, 1975; Karlsson, 1986) despite the effects of transpiration on plant water status and, consequently, on plant growth (Bradford and Hsiao, 1982).

In addition, specific studies on blue light are complex as this wavelength domain is also active on PAR-dependent mechanisms. In most studies, the lack of blue light was compensated for by saturating photosynthesis with red light backgrounds. Because of the higher relative response of photosynthesis to red light (McCree, 1972), such treatments did not allow photosynthetic and light quality effects on stomata to be separated. Moreover, Sager and colleagues (Sager *et al.*, 1982, 1988) have demonstrated that, under artificial light, the best indicator of photosynthetic utilization of a radiation source was not PAR level (or PFD; photosynthetic flux density) but photosynthetic efficiency.

Summarizing, our current understanding of the effect of blue light on stomatal functioning mainly comes from studies where blue light has been added (e.g. pulses). The opposite situation, i.e. when blue light is lacking or present at low levels, is also ecologically relevant as shaded zones

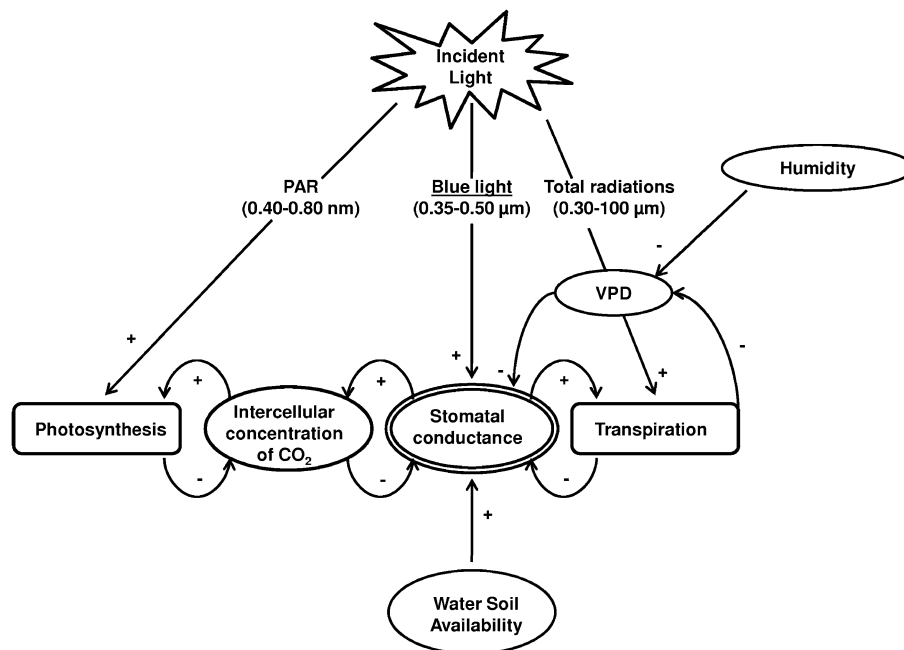


Fig. 1. Conceptual model of the stomatal control by environmental factors and consequences on gas exchange.

are characterized by the sudden attenuation of blue light. Nevertheless, there is little knowledge available on stomatal responses in these conditions. It might be supposed that low blue light levels with a constant PAR would lead to stomatal closure. However, the complex and numerous feedbacks and feedforwards (Fig. 1) that occur at the stomatal level lead us to believe that the stomatal closure induced by a low blue light level would trigger a decrease in the intercellular concentration of CO₂ which may, in turn, induce stomatal reopening.

This study focused on low blue light effects on stomata in order to quantify transpiration rate variations. Its objectives were (i) to characterize stomatal behaviour through leaf gas exchange measurement in response to a sudden and strong reduction in blue light, while maintaining relatively high PAR levels and equivalent photosynthetic efficiencies, and (ii) to test the putative role of *C_i* in blue light-induced gas exchange changes. Stomatal conductance, leaf transpiration, photosynthesis, and internal CO₂ concentration were recorded on mature tall fescue (*Festuca arundinacea*) leaves submitted to different light treatments and to a range of CO₂ concentrations.

Materials and methods

Plant material and growing conditions

Tall fescue clones (*Festuca arundinacea* Schreb. cv. Clarine) were planted in 0.4 l plastic pots filled with sand. Plants were grown in a cabinet at 80% relative humidity and were automatically watered eight times a day with a complete nutrient solution. The volume supplied to plants was varied from 30 ml d⁻¹ to 80 ml d⁻¹ according to their stage. Plants were grown under 380 μmol m⁻² s⁻¹ of PAR in a growth cabinet, with a 14 h photoperiod provided by metal halide lamps (HQI 400 W, Osram, France). Tall fescue clones were regularly produced in order to obtain plants at the same stage of development for gas exchange measurements.

Plants to be tested were transferred into a walk-in growth chamber for measurements where a 486 μmol m⁻² s⁻¹ PAR level was provided by metal halide lamps (HQI 400 W, Osram, France) with a 14 h photoperiod and a similar spectral composition to the growth cabinet.

The temperature in both growth cabinets was maintained at 19 °C both day and night.

Gas exchange measurements

Gas exchange measurements were performed using a portable infrared gas analyser (IRGA) (LI-6400; Li-Cor Inc, Lincoln, NE, USA) within a narrow leaf chamber (2×6 cm²; LI-6400-11). The top window was covered with Propafilm and had a PAR light sensor (GaAsp) beneath. The opaque base held a leaf temperature thermocouple (LI-6400-04). Stomatal conductance (*g_s*), leaf transpiration rate (*Tr*), leaf photosynthesis (*A*), and intercellular concentration of CO₂ (*C_i*), were then monitored in attached leaves under different light conditions and different CO₂ concentrations. Data stored by the LI-6400 were automatically corrected by leaf area corresponding to the leaf portion enclosed within the leaf chamber.

Light treatments and measurements

UVA-blue light is defined as radiation in the range of 350–500 nm. In this study, in order to avoid UVA effects, both the walk-in and growth chambers were equipped with a polycarbonate filter that

absorbed all radiation under 400 nm so that blue light was restricted to the range 400–500 nm.

The effects of low blue light on leaf gas exchange were studied by using light transmission filters on the top window of the leaf chamber. Low blue light levels (1.80 μmol m⁻² s⁻¹) were thus obtained with a Lee Filter HT 015 which also supplies high PAR levels (see detailed properties in Fig. 2A). The amount of transmitted PAR into the leaf chamber was calculated by using the optical properties of both transmission filters and the Propafilm fixed on the top of the leaf chamber (measured with the optical sphere and spectroradiometer Li-Cor 1800). The amount of blue light was calculated by using Equation 1 (de Berranger *et al.*, 2005):

$$BL = \int_{350}^{500} N_{\lambda, d\lambda} \quad (1)$$

where *BL* is the quantity of blue light (μmol m⁻² s⁻¹) and *N_λ* is the photon flux density in the wavelength λ (μmol m⁻² s⁻¹ nm⁻¹)

In view of the very low quantity of blue light, it was considered that blue light was lacking under this filter. A neutral filter (Lee filter 216) was used as a control providing a photosynthetic efficiency similar to the blueless PAR filter (Fig. 2A). The calculation of photosynthetic efficiency corresponds to an integration in the PAR wavelengths of the PFD times the relative quantum yield of each waveband (Equation 2; de Berranger *et al.*, 2005):

$$Y = \int_{300}^{780} N_{\lambda} \phi_{\lambda, d\lambda} \quad (2)$$

where *Y* is the photosynthetic efficiency (μmol m⁻² s⁻¹), *N_λ* is the photon flux density in the wavelength λ (μmol m⁻² s⁻¹ nm⁻¹), and φ_λ is the relative quantum yield of each waveband λ.

The Lee 216 filter provided a neutral shade as it lowered the energy from all wavelengths of the incident light by about 25%. The effects of blue light were then analysed by comparing the results between blueless and neutral filters.

Experimental protocol

Experiment 1: gas exchange response to blueless PAR: Gas exchange of the last fully expanded leaf was measured. Leaves were first placed in the gas exchange chamber under the white light of the walk-in growth chamber for 2 h of acclimation. Leaves were therefore allowed to reach a steady-state level of stomatal conductance for 2 h in order to achieve full stomatal activity. In this experiment, leaf temperature was set at 19 °C, the vapour pressure deficit (VPD) was fixed at 1 kPa, and the ambient CO₂ level at 400 μmol CO₂ mol⁻¹ (the level set in the reference chamber of the Li-Cor 6400). Each leaf was then submitted to a sequence of three light treatments (Fig. 2A): white light (ambient-W), neutral shade (N), and blueless PAR (B-). Stomatal conductance, leaf transpiration, leaf photosynthesis, and internal CO₂ concentration were recorded for 30 min under white light. Leaves were then submitted to the neutral light treatment (neutral light filter) for 90 min of monitoring. Finally, the blueless PAR filter was used immediately after the neutral treatment and gas exchange was measured for 60 min. Measurements were made on two sets of five plants. Gas exchange was first recorded on plants that reached the stage of three mature leaves on the main tiller and then on a second set of more developed plants: four to five mature leaves on the main axis and eight tillers (measurements were made on the axial tiller).

Experiment 2: interaction between blueless PAR and intercellular CO₂ concentration: In the second experiment (Fig. 2B), interactions between blueless PAR and intercellular CO₂ concentration and their effects on stomatal conductance were studied. Gas exchange of the last fully expanded leaf was measured. Leaves

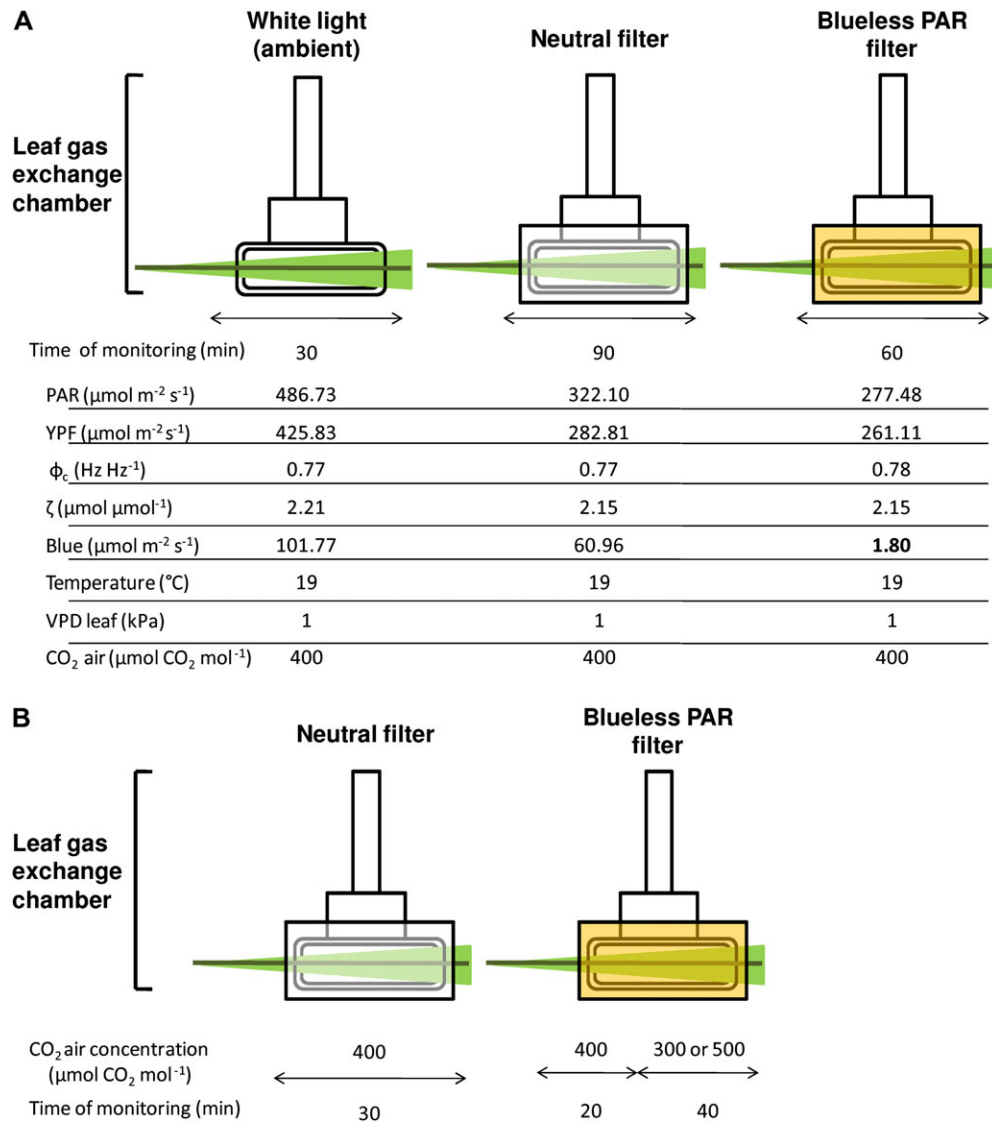


Fig. 2. Experimental protocol. Light treatments were obtained by placing light transmission filters on the top window of the leaf chamber. Environmental parameters of each condition are specified. PAR, photosynthetically active radiation; YPF, yield photosynthetic efficiency; ϕ_c , phytochrome photoequilibrium; ζ , zeta red:far red ratio; VPD leaf, vapour pressure deficit; the UVA-blue domain was defined between 400 nm and 500 nm. (A) Experiment 1. Gas exchange responses to the light treatment. Leaves were first placed in the gas exchange chamber under the white light of the walk-in growth chamber. Each leaf was submitted to the sequence of three light treatments: white light (ambient), neutral shade, and blueless PAR. Results were obtained from a set of young plants ($n=5$) and then from more developed plants ($n=5$). (B) Experiment 2. Interaction between blueless PAR and intercellular CO₂ concentrations. Leaves were put into the gas exchange chamber straight under the neutral filter. The blueless PAR filter was then placed on the gas exchange chamber for 60 min. In order to modify the C_i , two levels of CO₂ air concentration were imposed (either 500 or 300 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) 20 min after the beginning of the low-blue light treatment. Except for the CO₂, all of the other environmental conditions were maintained as described in Fig. 2A. Two plants were either submitted to an increase or a decrease of the CO₂ air concentration.

were then put into the gas exchange chamber under the neutral filter for 2 h of acclimation. Leaf temperature, VPD, and CO₂ concentration were fixed in the same way as in the first experiment. Leaf gas exchange was then recorded for 30 min under the neutral filter. The blueless PAR filter was finally placed on the gas exchange chamber for 60 min. Two levels of CO₂ (either 300 or 500 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) were then imposed for 20 min after the start of the blueless PAR treatment. This experiment was repeated on four plants from the first set (younger ones): two experienced a decrease of ambient CO₂ to 300 $\mu\text{mol CO}_2 \text{ mol}^{-1}$, and two an increase to 500 $\mu\text{mol CO}_2 \text{ mol}^{-1}$.

Statistical analysis

All statistical analyses were conducted in SAS 8.01 (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was performed with the GLM procedure, in order to determine whether or not light treatment had a significant effect in g_s , Tr , A , and C_i . Homocedasticity was verified by the random distribution in the residuals' plot for all variables. Comparisons of g_s , Tr , A , and C_i values among the light treatments (neutral versus blueless PAR) and among the different periods were performed using Scheffe's method (Steel and Torrie, 1980). The significance threshold was

fixed at the 0.05 probability level (α) for all statistical tests. The assumption that residuals were normally distributed with a mean of zero was also verified for all variables.

The dynamics of stomatal conductance in response to treatments (light or CO_2) were fitted by two non-linear models.

(i) Stomatal closure was fitted by the exponential decrease function:

$$g_s = g_{s0} e^{-k_c t} \quad (3)$$

where g_{s0} represents the stomatal conductance at the beginning of the blueless PAR treatment, k_c is a time-inverse parameter, and t is time. The highest rate of stomatal closure occurred at t_0 .

(ii) Stomatal reopening was fitted by the function:

$$g_s = g_{s_{\max}} (1 - e^{-k_o t}) \quad (4)$$

where $g_{s_{\max}}$ represents the maximum stomatal conductance (asymptote), k_o is a time-inverse parameter, and t is time.

Non-linear models were fitted using the least squares method (Steel and Torrie, 1980). Parameters were optimized using the Levenberg–Marquardt iterative method with automatic computation of the analytical partial derivatives. Initial seed values for the parameters depended on the variable being fitted (Escobar-Gutiérrez *et al.*, 2009).

Results

Blueless PAR effects on stomatal conductance and transpiration

A typical g_s response to light treatment in a younger plant is shown in Fig. 3A. This figure allowed the g_s kinetic to be divided into four periods in order to quantify the stomatal response among plants: (i) g_s initial values under the white light of the growing chamber (W), (ii) g_s response to the neutral treatment which represents the control (N), (iii) the maximum effect of the blueless PAR treatment, i.e. minimal g_s values (B–), due to a stomatal closure, and (iv) the stomatal reopening which led to new steady-states (B–ss). The overall data were then compiled into histograms in Fig. 4A (open bars) according to the periods defined previously.

Under white light (W), the stomatal conductance in younger plants was $0.39\text{--}0.55 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$. The neutral light filter application (N) decreased g_s by $13.7 \pm 3.7\%$ and g_s values remained stable afterwards. By contrast, blue light reduction (B–) to $1.80 \mu\text{mol m}^{-2} \text{ s}^{-1}$ triggered, in all of the measured leaves, a transient drastic and instantaneous (in the order of 1 min) decrease of g_s

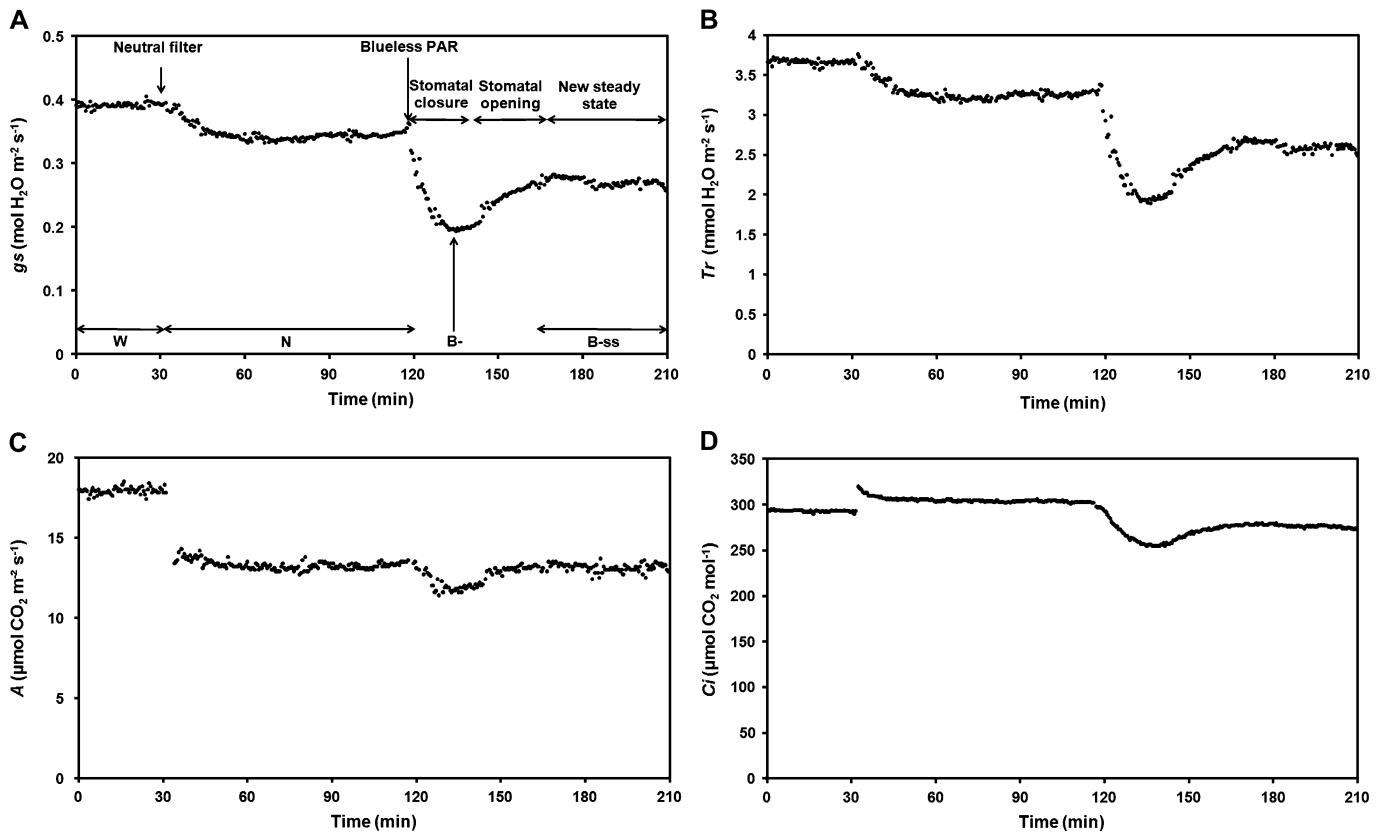


Fig. 3. Typical responses of stomatal conductance (A), transpiration rate (B), assimilation rate (C), and intercellular CO_2 concentration (D) to the light treatments in a younger plant. Leaves were first placed under white light (W: PAR= $486 \mu\text{mol m}^{-2} \text{ s}^{-1}$, Blue= $101 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and were then submitted to a neutral shade (N: PAR= $322 \mu\text{mol m}^{-2} \text{ s}^{-1}$, Blue= $61 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Finally, blue light was reduced to $1.80 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (PAR= $277 \mu\text{mol m}^{-2} \text{ s}^{-1}$) while maintaining an equivalent photosynthetic efficiency. Distinction was made between minimal values reached under blueless PAR treatment: B– (maximum effect of the blueless PAR treatment) and the new steady-states reached 45 min after blue light reduction (B–ss).

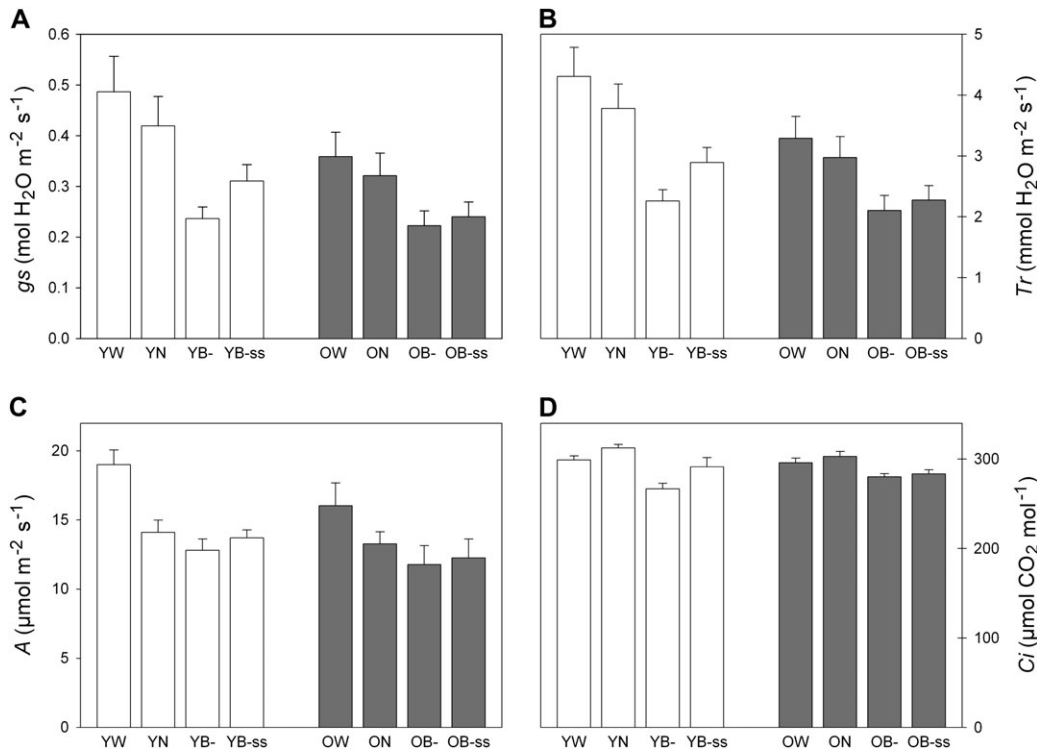


Fig. 4. Leaf gas exchange responses to the different light conditions in young (Y) and older (O) plants. Stomatal conductance (A), transpiration rate (B), assimilation rate (C), and intercellular CO₂ concentration (D) were measured under ambient light=white light (W: PAR=486 μmol m⁻² s⁻¹, Blue=101 μmol m⁻² s⁻¹), neutral shade (N: PAR=322 μmol m⁻² s⁻¹, Blue=61 μmol m⁻² s⁻¹), and blueless PAR for transient (B-) and steady-states (B-ss) responses (PAR=277 μmol m⁻² s⁻¹, Blue=1.80 μmol m⁻² s⁻¹). n=5 for both sets of plants. Results are mean values ±SD.

(Fig. 3A) by 43.2±4.5% compared with the neutral treatment ($P < 0.0001$; Table 1A). A minimal stomatal conductance of $0.24 \pm 0.02 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ was reached 19±2 min after blue light was withdrawn, after which a progressive increase in g_s occurred into the blueless PAR treatment. g_s values then stabilized (B-ss) at $0.31 \pm 0.03 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, 45 min after the start of the blue light reduction, at 74.5±5.5% of values measured under the neutral filter. It was therefore considered that g_s had reached a new and intermediate steady-state level significantly different from both the neutral treatment and from the minimum within the blueless PAR treatment ($P < 0.0001$ and $P < 0.01$, respectively; Table 1A).

Similarly to g_s , transpiration rates (Tr) were highest under the ambient light treatment (Fig. 3B), ranging from 3.66 to 4.91 mmol H₂O m⁻² s⁻¹ (Fig. 4B, open bars). The application of the neutral treatment decreased the transpiration rate by $12.1 \pm 3.3\%$ which then stabilized at $3.78 \pm 0.36 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. Under the blueless PAR treatment, Tr significantly decreased by $40.0 \pm 4.3\%$ compared with the neutral treatment ($P < 0.0001$; Table 1A) and then reached a minimal value of $2.26 \pm 0.17 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. Tr then increased and became stabilized 45 min after the blue light reduction, at $76.8 \pm 4.7\%$ of the transpiration rate observed under the neutral conditions. A new steady-state level was reached as B-ss values were significantly different from the neutral and the minimum within the blueless

Table 1. Results of the ANOVA test (P values).

(A) Comparisons performed in less developed plants (Y) and (B) in more developed plants (O). Statistical tests were performed between stomatal conductance (g_s), transpiration rate (Tr), assimilation rate (A), and intercellular concentration of CO₂ (C_i) measured under the neutral treatment (N), the blueless PAR maximum effect (B-), and when new steady-states (B-ss) were reached. Ns, no statistical difference. $n = 5$ for both younger and older plants.

(A)		YB-	YB-ss
YN	g_s	<0.0001	<0.0001
	Tr	<0.0001	<0.0001
	A	<0.001	ns
	C_i	<0.0001	<0.0001
YB-	g_s		<0.01
	Tr		<0.001
	A		<0.01
	C_i		<0.0001
(B)		OB-	OB-ss
ON	g_s	<0.0001	<0.0001
	Tr	<0.0001	<0.0001
	A	<0.0001	<0.01
	C_i	<0.0001	<0.0001
OB-	g_s		ns
	Tr		<0.05
	A		<0.1
	C_i		<0.1

PAR treatment (B-) ($P < 0.0001$ and $P < 0.001$, respectively; Table 1A).

Responses of g_s (Fig. 4A, shaded bars), and Tr (Fig. 4B, shaded bars), were also measured on the set of older plants. Under ambient conditions g_s and Tr were $0.31\text{--}0.42$ mol H₂O m⁻² s⁻¹ and $2.92\text{--}3.76$ mmol H₂O m⁻² s⁻¹, respectively. Under white light, these values were significantly lower than those found in younger plants (statistical test not shown). g_s and Tr then decreased under neutral treatment by $10.5 \pm 1.5\%$ and $10.0 \pm 1.4\%$, respectively. The absence of blue light triggered a significant decrease in g_s by $30.7 \pm 3.8\%$ and Tr by $29.1 \pm 3.4\%$ ($P < 0.0001$ for both g_s and Tr ; Table 1B). Minimal g_s values were measured 26 ± 5.75 min (data not shown) after the blue light reduction and finally reached steady-state levels at $75.0 \pm 4.0\%$ and $76.7 \pm 3.7\%$ of the neutral treatment for g_s and Tr , respectively.

Absolute minimal values (B-) of g_s and Tr were not significantly different between the two sets of plants, i.e. whatever plant stage the transient response (B-) of stomata lead to similar levels of stomatal conductance. Despite similar stomatal behaviour, g_s and Tr responses to blueless PAR in older plants differ by their amplitude and by their response time. In these plants, g_s and Tr decreased by 30.7% and 29.1% in response to blueless PAR instead of 43.2% and 40.0% in younger plants. Response to blueless PAR was also shifted by 7 min in older plants. This stomatal closure (k_c) was conducted at 0.24% s⁻¹ in more developed plants instead of 0.33% s⁻¹ in the younger plants (Table 2). Stomatal reopening was also less marked in more developed plants as g_s values were not significantly different between B- and B-ss periods (Table 1B). g_s increased from 0.24 to 0.31 mol H₂O m⁻² s⁻¹ in younger plants ($+31.6\%$) whereas the stomatal reopening did not exceed 8.2% in older plants (from 0.22 to 0.24 mol H₂O m⁻² s⁻¹). The rate of stomatal reopening (k_o) was therefore only calculated in younger plants: $k_o = 0.10\%$ s⁻¹ (Table 2).

Blueless PAR effects on photosynthesis

A typical photosynthesis (A) response to light treatments, in younger plants, is shown in Fig. 3C, and data are summarized in Fig. 4C (open bars).

Table 2. Rates of stomatal movements in response to blueless PAR in younger and older plants

Values were calculated after fitting of two non-linear functions (cf. statistical analysis). k_c represents the rate of stomatal closure and k_o the rate of stomatal opening. Mean values are shown \pm SD. $n=5$ for each set of plants.

	Younger plants		Older plants
CO ₂ air concentration (μmol CO ₂ mol ⁻¹)	400	400	400
Parameter (% s ⁻¹)	k_c	k_o	k_c
Mean value	0.33	0.10	0.24
SD	0.06	0.01	0.06

Mean A under white light was 19.01 ± 0.94 μmol CO₂ m⁻² s⁻¹. The neutral treatment decreased the assimilation rate to 14.10 ± 0.79 μmol CO₂ m⁻² s⁻¹. Such a decrease of 4.91 μmol CO₂ m⁻² s⁻¹ seems to be related to the PAR reduction that occurred under the neutral filter (-165 μmol m⁻² s⁻¹; Fig. 2A). After 1 h of monitoring, the blueless PAR treatment was applied and triggered a slight decrease of the assimilation rate at 12.81 ± 0.73 μmol CO₂ m⁻² s⁻¹ ($P < 0.001$; Table 1A). After 45 min, leaf photosynthesis stabilized at 13.71 ± 0.51 μmol CO₂ m⁻² s⁻¹ and therefore regained levels close to those found under the neutral treatment (difference not significant between these two treatments; Table 1A).

Mean A measured in older plants (Fig. 4C, shaded bars) was 16.00 ± 1.47 μmol CO₂ m⁻² s⁻¹ under white light. Photosynthesis kinetics under neutral and blueless PAR filters were similar to those observed in younger plants and afterwards stabilized at 12.25 ± 1.23 μmol CO₂ m⁻² s⁻¹ although it did not regain levels close to those found under the neutral treatment ($P < 0.01$ between N and B-ss; Table 1B).

Blueless PAR effects on intercellular CO₂ concentration (C_i)

Contrary to other variables, C_i was not directly measured by the IRGA. C_i is an estimated value derived from CO₂ concentration, stomatal conductance to CO₂, and transpiration and assimilation rates.

Estimated intercellular CO₂ responses in younger plants are shown in Fig. 3D (typical response) and summarized in Fig. 4D (open bars). Under white light, mean C_i was 300.00 ± 4.20 μmol CO₂ mol⁻¹. An increase of 5% in C_i was observed in response to the neutral treatment. Such an increase could be explained by the reduction of the CO₂ assimilation rate which is greater (25% in younger plants) than the reduction of the CO₂ influx (13.7%) due to the decrease in PAR. By contrast, blueless PAR led to a rapid decrease of C_i to a minimal value of 267.00 ± 5.50 μmol CO₂ mol⁻¹ ($P < 0.0001$; Table 1A), linked to the strong stomatal closure that occurred under blueless PAR conditions. Then an increase was observed leading in a new steady-state, reached 45 min after the blue light reduction, at $92.0 \pm 2.7\%$ of C_i values measured under the neutral filter.

Figure 4D (shaded bars) shows the C_i response to light treatment in older plants. C_i level under white light was 295 μmol CO₂ mol⁻¹. The neutral filter application triggered an increase of C_i by $2.3 \pm 1.2\%$ which is about half that observed in younger plants. This could arise from the moderate decrease of photosynthesis that occurred under the neutral treatment compared with younger plants, thus leading to a greater CO₂ consumption. Under blueless PAR, C_i decreased by $7.5 \pm 1.4\%$ ($P < 0.0001$; Table 1B). No significant increase in C_i was found after 45 min of blueless PAR treatment ($P > 0.05$; Table 1B). This could be related to the stomatal reopening which was less marked in older plants.

Impact of different intercellular CO₂ concentrations on the blueless PAR stomatal response

The second experiment was only performed on younger plants which were submitted to different CO₂ levels. Typical *g_s* and *C_i* responses to each treatment are shown in Fig. 5. Under neutral treatment, mean *g_s* values were 0.38–0.43 mol H₂O m⁻² s⁻¹. Then the blueless PAR filter was applied and *g_s* and *C_i* responded as described previously (Fig. 3A). Stomatal closure rates of 0.29% s⁻¹ (Table 3) were in agreement with those calculated in the first experiment. CO₂ air concentration changes were then imposed when *g_s* values were minimal, i.e. 19 min after the beginning of the blueless PAR treatment, a value that was rounded up to 20 min. *g_s* immediately responded to a decrease of the CO₂ air concentration (Fig. 5A). In fact, decreasing the ambient CO₂ concentration from 400 μmol CO₂ mol⁻¹ to 300 μmol CO₂ mol⁻¹ triggered a strong stomatal reopening. Then *g_s* stabilized at levels close to those found under the neutral treatment, thus leading *C_i* to remain at sufficient levels in spite of the decrease by 100 μmol CO₂ mol⁻¹ of the CO₂ air concentration (Fig. 5B). Nevertheless, the stomatal reopening (*k_o*; Table 3) appeared to be slightly slower than that previously seen 20 min after the blue light reduction: 0.06% s⁻¹ instead of 0.10% s⁻¹ in these younger plants.

By contrast, an increase of CO₂ air concentration from 400 μmol CO₂ mol⁻¹ to 500 μmol CO₂ mol⁻¹ (according to the same protocol) triggered an opposite response (Fig. 5C, D). In that case, *g_s* further decreased in response to a higher CO₂ air concentration down to a value equivalent to 63.0±1.6% of that measured under the neutral filter without CO₂ modification. This step of stomatal closure was rapidly conducted with *k_c*=0.46% s⁻¹.

Discussion

Blueless PAR stomatal response and *C_i* involvement

The objective of this study was to quantify blueless PAR effects on stomatal conductance (*g_s*) and its consequences on transpiration (*T_r*) in tall fescue. Despite the variability of *g_s* absolute values (Fig. 4A), all leaves belonging to plants at the same stage exhibited similar *g_s* (data not shown). This demonstrates that *g_s* responses to the light treatment were similar and proportional to the initial conductance level as observed by Karlsson (1986). Further, a sudden blue light reduction from 60.96 μmol m⁻² s⁻¹ to 1.80 μmol m⁻² s⁻¹ triggered a transient drastic and instantaneous decrease of *g_s* whatever the plant stage. Similar results have been

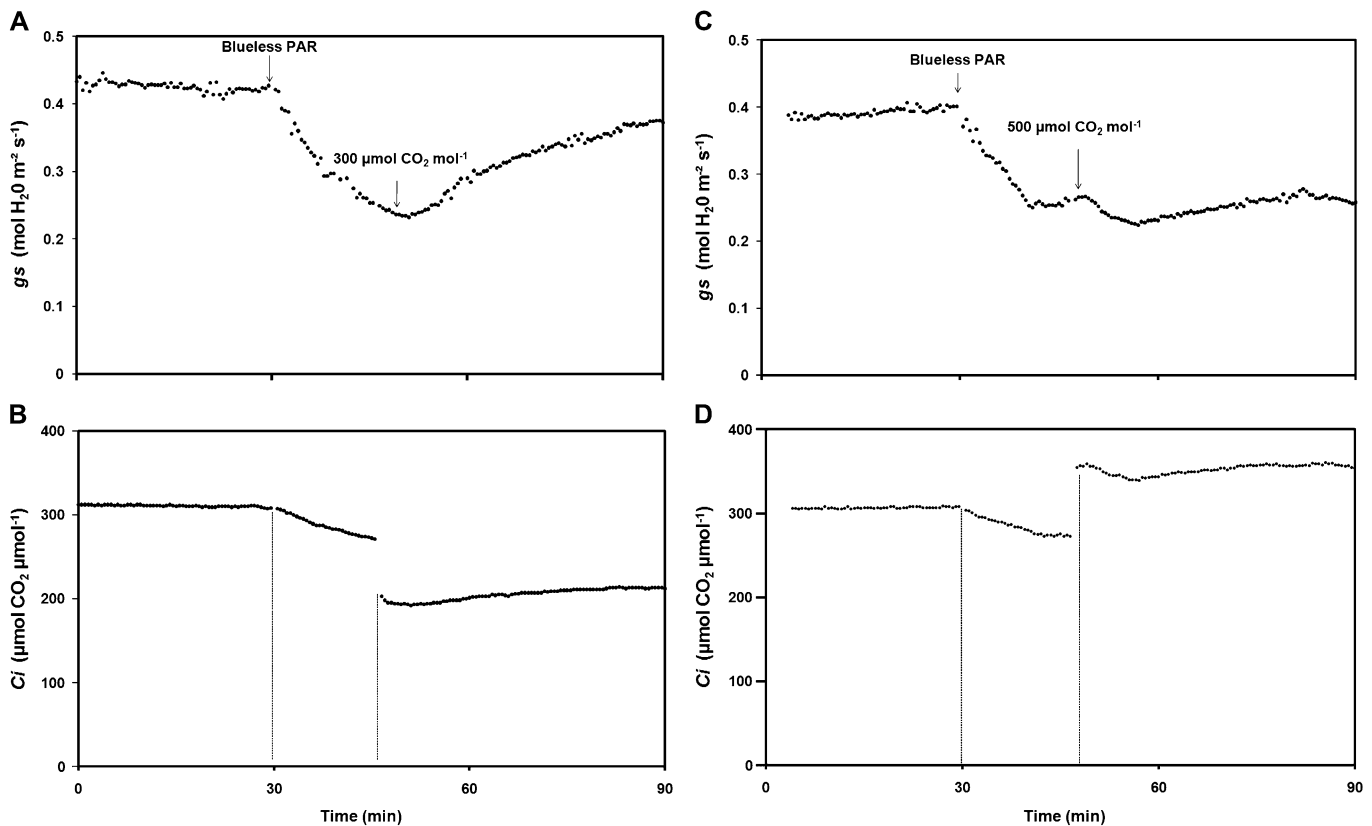


Fig. 5. Typical stomatal conductance (*g_s*, up) and intercellular concentration of CO₂ (*C_i*, down) response to blueless PAR and to CO₂ air concentration. Leaves were put into the gas exchange chamber straight under the neutral treatment (PAR=322 μmol m⁻² s⁻¹, Blue=61 μmol m⁻² s⁻¹) with 400 μmol CO₂ mol⁻¹. The blueless PAR treatment (PAR=277 μmol m⁻² s⁻¹, Blue=1.80 μmol m⁻² s⁻¹) was then applied, triggering a stomatal closure. (A, B) CO₂ air concentration was set to 300 μmol CO₂ mol⁻¹ 20 min after the blueless PAR treatment beginning. (C, D) CO₂ air concentration was set to 500 μmol CO₂ mol⁻¹ 20 min after the blueless PAR treatment began.

Table 3. Different CO₂ air concentration effects on the rate of stomatal opening (k_o)

CO₂ concentration changes were performed when the blueless PAR effect on stomatal conductance was maximal (B-). k_c and k_o values were calculated after fitting of two non linear functions (cf. statistical analysis). $n=2$ for each treatment (300 and 500 $\mu\text{mol CO}_2 \text{ mol}^{-1}$).

Younger plants			
CO ₂ air concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	400	400 to 300	400 to 500
Parameter (% s ⁻¹)	k_c	k_o	k_c
Mean value	0.29	0.06	0.46
SD	0.04	0.01	0.03

reported by Zeiger (1984) in *Xanthium strumarium* and by Karlsson and Assmann (1990) in *Hedera helix* when blue light was switched off from a red light background. Stomatal responses under strongly or totally reduced blue light are poorly documented in comparison to the large body of literature dealing with the addition of blue light by pulses or continuous lighting (Iino *et al.*, 1985; Karlsson, 1986; Zeiger *et al.*, 1987; Assmann and Grantz, 1990). In these studies, an inverse kinetic, i.e. stomatal opening, was observed whatever the plant species and the range of blue light fluence rates tested, from 250 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (Iino *et al.*, 1985) to 1.1 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (Karlsson, 1986). Moreover, blue light was generally superimposed on red light backgrounds and/or in plants exhibiting particular developments in relation to their light environment (e.g. plants kept in darkness or hypocotyls). However, both blue and red wavelengths play an important role in photomorphogenesis and photosynthesis, so that, in these studies, PAR-dependent responses of stomata to blue light could not be excluded (McCree, 1972; Zeiger, 1984).

In order to separate PAR-dependent and photomorphogenic responses, light transmission filters were used that ensure an equivalent photosynthetic efficiency and exhibit similar properties for the phytochrome photoequilibrium (ϕ_c) and the R:FR ratio (ζ), as reported in Fig. 2A. As expected, steady-state levels of assimilation rate measured under the blueless PAR condition were close to those found under the neutral treatment (Fig. 4). On the other hand, cutting the supply of blue light strongly reduced g_s that, surprisingly, did not stay at its minimal values but reached a steady-state at an intermediate level (Fig. 4A), particularly in younger plants. It was hypothesized that this stomatal reopening may be in relation to the constant photosynthetic demand related to the electron flow that occurred under the blueless PAR treatment. This treatment triggered a rapid stomatal closure which reduced the CO₂ uptake and thus C_i (Fig. 5). Once CO₂ stocks are consumed by photosynthesis, one or more signals may therefore induce stomatal reopening thus allowing the leaf to maintain a constant assimilation rate. However, g_s did not regain its initial levels observed under the neutral filter but reached a new stomatal equilibrium which may be balanced between the blueless PAR signal (closure) and the photosynthetic demand

through C_i (opening). The results from the C_i level manipulation confirmed the potential implication of C_i in the stomatal behaviour in response to blueless PAR, in particular, for stomatal reopening. In fact, a reduction of external CO₂ concentration during the blueless PAR treatment triggered a strong stomatal reopening thus allowing leaves to maintain sufficient C_i to ensure constant photosynthesis (Fig. 5B). By contrast, an increase of external CO₂ concentration triggered an additional stomatal closure that enhanced the blueless PAR effect as C_i was no longer limiting (Fig. 5D). These results strengthened the notion of CO₂ stomatal control as reported by Morison and Gifford (1983), which would modulate the blue light response of stomata (Lasceve *et al.*, 1993). Further, the steady-state response of g_s to C_i could be controlled by photosynthetic electron transport which is therefore sensitive to the balance between the light and dark reactions of photosynthesis (Messinger *et al.*, 2006). Moreover, the amplitude of stomatal responses involves signal exchanges, other than C_i , between the mesophyll and epidermal cells, including guard cells (Mott, 2009).

Blueless PAR perception and possible molecular mechanisms

According to the literature, stomatal responses to blue light could be mediated by photoreceptors located in the stomata, for example, phototropins which are involved in photomovement. In addition, other studies have proposed zeaxanthin, located in guard cell chloroplasts, as a molecule having the dual function of a blue light and a CO₂ sensor that mediates blue-light-specific stomatal opening (Srivastava and Zeiger, 1995a, b; Zeiger and Zhu, 1998; Zhu *et al.*, 1998). Two main mechanisms have so far been identified. First, these photoreceptors may control proton extrusion (Raschke and Humble, 1973). We could therefore hypothesize that low blue light may inactivate plasma membrane H⁺-ATPase thus leading to stomatal closure (Shimazaki *et al.*, 2007). More recently, Vahisalu and colleagues (2008) have also identified the *SLAC1* gene, preferentially expressed in guard cells, that encodes an essential subunit for S-type anion channels. These channels seem to function as central regulators of stomatal closure induced by several factors such as light, CO₂, humidity, and ABA (Keller *et al.*, 1989; Schroeder and Hagiwara, 1989; Vahisalu *et al.*, 2008). Thus, in our study, the low blue light conditions could, through these channels, activate an anion efflux and cause membrane depolarization (which controls K⁺ channels) and finally induce stomata closure.

Variability of stomatal responses to blue light

It has been shown that the magnitude of the stomatal responses to blue light could depend on environmental factors such as CO₂ concentration but it could also differ between and within plant species. Indeed, Loreto *et al.* (2009) have shown that stomatal conductance in *Platanus* and *Nicotiana* leaves is relatively insensitive to blue light

increase whereas, in general, it stimulates stomatal functioning (Zeiger, 1984). These results could be explained by the experimental conditions because blue light was changed from 0% to 80% of PAR (fixed at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$) thus modifying the energy balance and photosynthetic efficiency components. Consequently, the PAR-dependent effects on stomatal conductance discussed above cannot be excluded. This hypothesis is strengthened by the fact that the apparent stability of stomatal conductance was explained by changes in mesophyll conductance, partly due to chloroplastic rearrangements.

In our study, all *Festuca* leaves tested exhibited similar stomatal behaviour in response to low blue light. However, leaves of more developed plants exhibited delayed and less marked g_s decreases to blueless PAR. This behaviour highlights the importance of plant developmental stage on blue light stomatal sensitivity which could be explained by (i) an 'age effect' (Field, 1987) and/or by (ii) the important nutritive needs of more developed plants and, in particular, related to water status. Hormonal signals could therefore be involved, for example, abscisic acid (ABA) that also controls stomatal closure (Raschke, 1987; Roelfsema and Hedrich, 2005). As a consequence, variations in blue light stomatal responses, observed within a stand, could be related not necessarily to intraspecific genetic variability but to differential blue-light sensitivities, which are therefore dependent on the ontogenic development.

Blueless PAR effects at stomatal scale: a link with the blue light modulation of leaf growth through leaf transpiration?

In this study it has been demonstrated that blueless PAR triggers a rapid stomatal closure followed by a decrease of leaf transpiration by 41.1%. This is consistent with other studies although the inverse response was described, i.e. blue light pulses that triggered transpiration rate increases (Brogardh, 1975; Johnsson *et al.*, 1976; Karlsson, 1986). Moreover, low blue light is also known to enhance leaf growth (Gautier and Varlet-Grancher, 1996) independently from PAR level (Christophe *et al.*, 2006). Several hypotheses which are not exclusive could be put forward in order to explain this enhanced growth by low blue light levels. On the one hand, biochemical and biomolecular mechanisms could be involved, for example, blue light effects on auxin transport (Thornton and Thimann, 1967), on cell division (Munzner and Voigt, 1992) or on cell wall extension (Folta *et al.*, 2003). On the other hand, blue light effects on leaf growth could be approached through its effects on stomatal conductance and therefore on transpiration. In fact, Martre *et al.* (2001) and Parrish and Wolf (1983) showed that leaf growth and leaf transpiration are highly correlated. Such a link between blue light, growth, and water status has also been made by Cosgrove and Green (1981). These authors demonstrate that an addition of blue light strongly inhibits hypocotyl growth by decreasing the yielding properties of cell walls and thus modifying cell turgor pressure.

Although the present work was conducted at the leaf scale, extrapolations at the whole plant level within a stand remain ecologically coherent as shaded zones could be localized just affecting small parts of the plant. Such work would nevertheless require further experiments at the plant scale coupled with modelling approaches in order (i) to establish the quantitative relationships between stomatal responses and actually perceived blue light and (ii) to confirm the hypothesis of a whole plant transpiration modulation by blue light and to quantify hydric pathway involvement (through stomata) in the regulation of leaf growth by blue light.

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