Sensitivity to far-red radiation in stomata of *Phaseolus vulgaris* L.: Rhythmic effects on conductance and photosynthesis

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Abstract. The influence of far-red (FR; 700–800 nm) radiation on steady-state stomatal conductance and net photosynthesis in *P. vulgaris* has been studied. Whereas FR radiation alone was relatively ineffective, addition of FR to a background of white light (WL; predominantly 400–700 nm) resulted in increased stomatal conductance. Stomata exhibited a marked diurnal sensitivity to FR. The action maximum for enhancing stomatal conductance was near 714 nm. A combination of FR and infra-red (IR; >800 nm) enhanced net photosynthesis when added to a background of WL. When IR alone was added to WL, there was a net decrease in photosynthesis, indicating that it is the FR waveband which is responsible for the observed photosynthetic effects. Naturally occurring levels of FR radiation (235 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) in vegetation-canopy shade enhanced net photosynthetic CO\(_2\) gain by 28% when added to a background of 55 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) WL.

Key words: *Phaseolus* (photosynthesis, stomatal opening) – Photosynthesis (active radiation) – Phytochrome and stomatal opening – Stomatal opening – Transpiration.

Introduction

Light is an important environmental factor in the regulation of stomatal movement in plants (see e.g. Zeiger 1983 for review). On an equal photon basis, blue (BL; 400–500 nm) is more effective than red (R; 600–700 nm) in causing stomatal opening, and other wavebands have been reported to be relatively ineffective (Kuiper 1964; Hsiao et al. 1973; Sharkey and Raschke 1981). There is general agreement in the literature that BL radiation operates primarily through one or more specific BL-absorbing photoreceptors, whereas the R response is photosynthetic (Raschke 1975; Sharkey and Raschke 1981; Zeiger 1983). These conclusions have been derived from studies using monochromatic radiation, or dichromatic treatments with BL and R radiation.

Holmes and Klein (1985) reported that pre-irradiation of *Phaseolus vulgaris* L. leaves with far-red (FR; 700–800 nm) increases the rate of stomatal opening under BL radiation and the rate of closure in subsequent darkness. Both responses were mediated through phytochrome. We have recently observed long-term enhanced stomatal opening under continuous white light (WL; predominantly 400–700 nm) if this was supplemented by FR radiation. This continuous response to FR could not be ascribed to phytochrome. We present experiments with primary leaves of *P. vulgaris* which were designed to determine whether the increased steady-state stomatal opening in response to supplementary FR may be associated with a photosynthetic response to FR. We have also attempted to distinguish between any direct photochemical effects of FR on photosynthesis and indirect effects of temperature resulting from infra-red (IR; >800 nm) radiation, and to relate the observations to their possible consequences for plants growing under natural conditions.

Material and methods

Plant material, *Phaseolus vulgaris* L., cv. Provider (Meyer Seed Co., Baltimore, Md., USA) were sown and grown at 23° C,
50 ± 7% relative humidity, in vermiculite over an approx. 10-mm-thick layer of gravel. Tap water was used at sowing and 0.5-strength Formula A1 Purdue nutriculture medium (Withrow and Withrow 1948) for subsequent irrigation. Measurements of diffusive conductance were made on primary leaves immediately prior to the expansion of the first trifoliate leaves, typically 15-18 d after sowing. Unless otherwise stated, the plants were subjected to 12:12-h light:dark cycles during the pre-treatment period, the light being provided by a bank of Sylvania (GET Sylvania, Danvers, Mass., USA) F48T12/D/VHO “Daylight” fluorescent lamps (100 μmol·m⁻²·s⁻¹ in the 400-700 nm waveband).

Chlorophyll-free plants were obtained by imbibing seeds for 24 h in 2.5·10⁻⁵ M Norflurazon (SAN 9789; 4-chloro-5-(methylamino)-2-(z,a,a-trifluoro-m-cyano-3(2H) pyridazinone; Sandoz-Wander, Homestead, Fla., USA) in distilled water. This herbicide inhibits carotenoid synthesis, thereby leading to chlorophyll photodestruction (Bartels and Hyde 1970). Plants from the imbibed seeds were then grown under the same conditions as the green plants except that the nutrient solution contained 2.5·10⁻⁵ M Norflurazon. These chlorophyll-free plants were used for experiments 12–16 d after sowing. Possible contamination of the leaves with chlorophyll was always checked under the fluorescence microscope at the end of each experimental run.

Stomatal opening and closing was estimated indirectly by measuring the diffusive conductance of primary leaves with two different types of porometer. For short-term measurements (Table 1, Figs. 3, 4a), an LI-1600 steady-state porometer (LI-Cor, Lincoln, Neb., USA) was used with a preset relative humidity of 50% as this was the relative humidity at which the plants had been grown. A circular aperture (200 mm²) was used to measure diffusive conductance of the abaxial surface (Table 1). A Li-Cor 1600-02A cylindrical chamber was used to measure conductance of the entire leaf (i.e. both abaxial and adaxial surfaces) in the experiments described in Figs. 3 and 4a because a similar chamber was used for photosynthesis measurements. For experiments requiring long-term monitoring (Fig. 2), a modified Li-Cor LI-65 porometer was used (Holmes and Klein 1985); the cuvette was modified to carry out irradiation of leaves in situ, and continuous monitoring was provided by connecting the analog output of changes in conductance detected by the humidity sensor to a chart recorder. Qualitative comparison of conductance measurements of only the lower epidermis or both lower and upper epidermis is justified from preliminary studies which demonstrated that 91% of transpirational water loss takes place through the abaxial leaf surface of this cultivar. All instruments were calibrated regularly against standard LI-Cor calibration equipment.

Net photosynthesis and respiration were measured with an automated differential infra-red gas analyser (Analytical Development Co., Hoddesdon, Herts., UK). The system was operated on an open circuit in which CO₂ was mixed with CO₂-free air to provide CO₂ concentrations of 400 ± 20 μl⁻¹. CO₂ standards were used for calibration. Relative humidity was 70% and the flow rate through both reference cell and cuvette was 300 ml·min⁻¹. The cuvette was constructed of 3-mm-thick by 30-mm-diameter polycarbonate tubing split in half with 3-mm flat-end caps. The volume of the closed cuvette was 19.8 ml with a clamped leaf area of 840 mm². The leaf was clamped in the cuvette by spring tension and sealed by 3-mm-wide foam strip around the edges of the cuvette. The gas mixture was split to pass equally over both leaf surfaces. The complete system was controlled by an Apple-ISAAC acquisition and control system (Cyborg Corp., Woburn, Mass., USA) which automatically monitored the absolute concentration of CO₂ in the gas mixture and provided daily calibrations (both absolute and differential). The system produced statistical summaries of the differential CO₂ concentrations across the cuvette, leaf temperature (thin wire thermocouple), and monitoring of the flow rate at the cuvette surface (Li-Cor LI-200SB quantum sensor).

![Fig. 1](image-url) Spectral characteristics of the radiation sources used in the experiments for Figs. 3, 4, and 5. The WL for Fig. 3 (a; ----) was produced by the radiation from a xenon arc (Leitz, Wetzlar, FRG) filtered through two 3-mm-thick Schott KG1 heat-absorbing filters and a Corion LG-400-S ultra-violet-absorbing filter. The monochromatic radiation for Fig. 3 (a; ----) was produced by filtering the output of a modified Withrow tungsten filament monochromator (Withrow 1957) through Bausch & Lomb (Rochester, N.Y., USA) or Corion (Holliston, Mass., USA) narrow bandpass (≤ 7 nm) interference filters and appropriate Schott & Gen. (Mainz, FRG) cut-on filters; the broadest filter (central wavelength = 710 nm) is given as an example. The WL for Fig. 4 (b; ----) was derived from the Leitz xenon arc source filtered through a 3-mm Schott KG1, and a Corion LG-400-S filter; WL was attenuated with Kodak (Eastman Kodak Co., Rochester, N.Y., USA) neutral density gelatin filters. Added FR in Fig. 4 (b; ----; 366 μmol·m⁻²·s⁻¹) was obtained by filtering the radiation from a modified Withrow tungsten filament source through 10 cm of water containing 1.5% (w/v) ferrous ammonium sulphate, one 3-mm KG1 filter and a Corion LG-697-S cut-on filter. The WL in Fig. 5 (e; ----) was produced by the Leitz xenon arc source filtered through a 3-mm Schott KG4 heat-absorbing filter, a Corion LG-400-S, and two Corion 705-nm cut-off (longer-wavelength-blocking) filters; fluorescence rate was adjusted with Kodak neutral density gelatin filters. FR + IR in Fig. 5 (e; ----) = Withrow tungsten filament source + 3-mm Schott KG4 filter + Corion LG-697-S cut-on filter. IR in Fig. 5 (e; ----) = Withrow tungsten filament source + 3-mm Schott KG4 + Corion LG-790-S cut-on filter.