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Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanulas

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ABSTRACT

To investigate the effect of the light spectrum on photosynthesis, growth, and secondary metabolites *Rosa hybrida* 'Scarlet', *Chrysanthemum morifolium* 'Coral Charm', and *Campanula portenschlagiana* 'BluOne' were grown at 24/18 °C day/night temperature under purpose-built LED arrays yielding approximately 200 μmol m^{-2} s^{-1} at plant height for 16 h per day. The four light treatments were (1) 40% Blue/60% Red, (2) 20% Blue/80% Red, (3) 100% Red, and (4) 100% White (Control). The plant height was smallest in 40% Blue/60% Red in roses and chrysanthemums, while the biomass was smallest in the white control in roses and in 100% Red in chrysanthemums. The total biomass was unaffected by the spectrum in campanulas, while the leaf area was smallest in the 40% Blue/60% Red treatment. In 100% Red curled leaves and other morphological abnormalities were observed. Increasing the blue to red ratio increased the stomatal conductance though net photosynthesis was unaffected, indicating excess stomatal conductance in some treatments. With higher blue light ratio all phenolic acids and flavonoids increased. In view of the roles of these secondary metabolites as antioxidants, anti-pathogens, and light protectants, we hypothesize that blue light may predispose plants to better cope with stress.

Introduction

In many greenhouses in Northern European countries, supplemental lighting is used from fall to spring, to enhance plant growth and to obtain all year round high production and good quality plants. In commercial practice, greenhouse plants are supplied with supplementary light for up to 16 h per day (while in Denmark up to 20 h photoperiod is used) and the light intensity ranges between 100 and 200 μmol m^{-2} s^{-1} (Paradiso et al., 2011). High pressure sodium (HPS) lamps are the most common lighting source; however they seem to be neither spectrally nor energetically optimal (Marcelis et al., 2006). Manipulation of the lighting spectrum of the lamps could trigger potential benefits for the plants (Brazaitytė et al., 2006), but none of the HPS lamps provide the possibility for spectral manipulation. Recently, light emitting diodes (LEDs) have been introduced for supplemental lighting, as they might have a variety of advantages compared to the traditional lighting systems: longer lifetime, smaller size, lower heat emission, and higher energy conversion efficiency (Massa et al., 2008). The use of LED luminaries has the potential of passing significant savings to greenhouse growers. The LED lighting has yet to be fully integrated within the greenhouse control system and should be optimized in terms of light output, while LED luminaire cost should be reduced in order to reach a sustainable and economically viable production (Morrow, 2008). However, the current costs of LED luminaries, the lack of extensive trials, and the diversity of horticultural species have not led to full adoption also due to the fact that many luminaries have not been properly documented. Integration of LEDs in current growing systems receives full attention as they provide the opportunity to control the light spectrum to some degree (Whitelam and Halliday, 2007; Trouwborst et al., 2010). The use of red LEDs was initially accepted since the red wavelengths (600–700 nm) are efficiently absorbed by plant pigments, such as chlorophyll, which has one of the absorption peaks at 665 nm (Sager and McFarlane, 1997); current commercial lamps are usually based on a 80% red and 20% blue light combination.

Abbreviations: g_s, stomatal conductance; HPLC, high pressure liquid chromatography; HPLC–PDA, high pressure liquid chromatography–photodiode array detector; HPS, high pressure sodium; LC–MS, liquid chromatography–mass spectrometry; LA, leaf area; LEDs, light emitting diodes; P_n, net photosynthesis; SMs, secondary metabolites; ROS, reactive oxygen species; UV, ultraviolet.

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The full visible spectrum drives the photosynthetic metabolism, while far-red light in combination with red is primarily responsible for germination, elongation, and flowering (Saebø et al., 1995). Plants cannot optimally develop with monochromatic red light alone, but need blue and far-red as well to regulate other types of responses. Blue light is also responsible for vegetative growth (Terfa et al., 2012a,b). The amount of blue light required by different species is an on-going discussion; however the importance of blue light was reported early as it plays an important role in the photomorphogenetic properties of the plants and stomatal control which affect water relations, CO_2 exchange, stem elongation, and phototropism (Hogewoning et al., 2010; Islam et al., 2012).

Little is known about the physiological relationship between photosynthesis and secondary metabolism for ornamental greenhouse plants grown under LEDs, irrespective of whether they are grown in closed rooms or under natural light in greenhouses. Plants use part of their energy to synthesize secondary metabolites (SMs). Despite this energy burden they have been retained throughout the plant kingdom. This is probably because the high diversity and vast variety of SMs have evolved as a means for plants to interact with the environment and for the protection against biotic and abiotic stress (Lattanzio et al., 2006). A wide range of external stimuli trigger changes in the plant cell and SMs are produced to overcome the stress factors. Ultraviolet (UV) radiation is an essential ecological factor affecting organisms and ecosystems and is connected to the changes plants have undergone throughout the development of life on earth (Hideg et al., 2013). Blue and UV-A light share the same photoreceptors (cryptochromes and phototropins) which absorb at a range from ≈320 to 500 nm (Cashmore et al., 1999; Lin and Shalitin, 2003). Therefore, it is pertinent to investigate the effect of blue light on the production of SMs from a lighting point of view focused on LEDs.

Phenolic acids and flavonoids are used by plants for pigmentation, resistance to pathogens, as well as a defence mechanism under stressful conditions (Shetty et al., 2011). Different SMs absorb in different parts of the UV and visible region of the spectrum, protecting the plant from excessive radiation (Gläβgen et al., 1998; Lattanzio et al., 2006). Earlier research showed that changes in light spectrum could result in changes in the amount of SMs (Li and Kubota, 2009).

We hypothesized that: (1) varying the ratio between red and blue light would trigger the protection mechanisms of the plants and (2) the effects of different spectra from LEDs will vary among species. The objectives of this study were to characterize: (1) the effect of spectral composition on the photosynthetic and morphological characteristics of roses, chrysanthemums, and campanulas and (2) the effect of different spectra at uniform irradiance on the amount of phenolics and flavonoids in the selected species when plants were grown with low level of natural light.

Materials and methods

Plant material and growth conditions

The plants were grown in greenhouses at Aarhus University, Aarslev, Denmark (lat. 55.309° N, long. 10.439° E) from November 2011 to January 2012 using potted *Rosa hybrida* 'Scarlet', *Chrysanthemum morifolium* 'Coral Charm', and *Campanula portenschlagiana* 'BluOne'. The experimental design was a randomized complete block design with sub blocks consisting of four light treatments. Plants were grown under four purpose-built LED arrays (research modules, Philips, Eindhoven, NL) yielding approximately 200 $\mu mol\,m^{-2}\,s^{-1}$ for 16 h per day. The natural light was limited due to short daylight period as well as from internal shading from installed screens and lamps. The daily light integral was measured at 11.52 $mol\,m^{-2}\,d^{-1}$. The wavelengths of blue and red LEDs used are 450–485 nm and 650–670 nm, respectively. The four LED light treatments were (1) 40% Blue in 60% Red light (40%B/60%R), (2) 20% Blue in 80% Red (20%B/80%R), (3) 100% Red (100%R), and (4) 100% White light (Control). The white LED light contained 32% blue (400–500 nm), 46% green (500–600 nm), and 22% red light (600–700 nm). The HPS lamps were not used as a Control as the main focus of the study was to compare treatments grown under natural and supplementary LED lighting only. Sub-blocks were randomly assigned at the beginning of the experiment. The temperature in the greenhouse compartments was set to 24 °C and 18 °C during the day and night, respectively and the actual temperature was very close to the set point due to the winter conditions and low natural irradiance. The plants were grown to flowering (except chrysanthemums as the long day conditions prevented flower induction) and plant growth was recorded at the end of the experiment. Fresh and dry weight of the stems and leaves, leaf area (LA), plant height, and number of buds (when appropriate) were recorded.

Photosynthetic characteristics

The photosynthetic characteristics [net photosynthesis (P_n) and stomatal conductance (g_s)] were measured by light response curves using the CIRAS-2 portable system with leaf cuvette PLC6 and standard LED unit (U) measuring a LA of 2.5 cm^2 (PP Systems, Amesbury, MA, USA). The photon flux densities used were 0, 50, 100, and 200 $mmol\,m^{-2}\,s^{-1}$. Four leaves per plant were used for measurements. Before the measurements were taken, the leaf was held within the cuvette until a steady state of photosynthesis and g_s was reached. The measurements were performed between 9 am and 2 pm to ensure a fully photosynthetically active plant. The spectrum of the LED unit (CIRAS2 Portable Photosynthesis System, PP Systems, Amesbury, MA, USA) fitted with LXHL-BD03 and LXHL-BW03, LUXEON® (Philips Lumileds Lighting Company, The Netherlands) with a mix of 80% red and 20% cold white (blue).

Secondary metabolites

Ten leaves from roses, five from chrysanthemums, and 15 from campanulas were taken randomly and stored at −80 °C for later analysis by high pressure liquid chromatography (HPLC). Approximately 1 g of roses, 3 g of chrysanthemums, and 1 g of campanulas were ground with liquid nitrogen and 10 mL of 80% methanol (MeOH, VWR International, Herlev, Denmark) was used for extraction. All samples were extracted in darkness for at least 90 min and 1 mL was filtered on 0.2 μM micro filters (Whatman GmbH, Dassel, Germany) and put into HPLC vials; all samples were kept at −80 °C until later analysis with HPLC (Shetty et al., 2011). Extracts were analyzed by high pressure liquid chromatography–photodiode array detector (HPLC–PDA) on a Dionex-Chromeleon Chromatography Data System (Thermo Scientific™ Dionex™ Chromeleon™ 7.1 Chromatography Data System, Sunnyvale, CA, USA). The software used for interpreting the results of the liquid chromatography–mass spectrometry (LC–MSMS) was Xcalibur version 2.0.7. Separations were performed on a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm; Agilent, Santa Clara, CA, USA) with the following solvents: solvent A = 0.1% formic acid (HPLC grade, purity of 99%; Sigma–Aldrich, Denmark A/S, Copenhagen, Denmark) in water and solvent B = 0.1% formic acid in acetonitrile (HPLC grade; Fisher Scientific, Waltham, MA, USA). The column was maintained at 30 °C using a thermostated column compartment. The HPLC method for the analysis of roses had a run time of 76-min and the flow was 1.0 $mL\,min^{-1}$. The solvent gradient was from 0 to 5 min isocratic 1% B, from 5 to 15 min isocratic 12% B, from 15 to 30 min linear gradient from 12 to 40% B, from 30 to 35 min linear gradient from