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Species-specific differences in synthesis of flavonoids and phenolic acids under increasing periods of enhanced blue light

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ABSTRACT

Species-specific differences in synthesis of flavonoids and phenolic acids were studied under lengthening periods of enhanced blue light in a greenhouse experiment in Northern Finland in the autumn of 2012. The aim was to compare red- and blue- weighted light spectra in relation to biosynthesis of the compounds. The species studied were red leaf lettuce and basil. There were five treatments for these inter-lighting LED manipulations using traditional high pressure sodium lamps as background light sources. Two treatments were exposed for the entire experimental period with (1) red- and (2) blue-weighted light for 48 days. The other three treatments were initiated with red- weighted light, but after each subsequent 12 day sub-period, one red-weighted treatment was switched to blue, resulting in the following treatments: 48, 36, 24, 12 and 0 days under enhanced blue light. Flavonoid and phenolic acid biosynthesis in plants were found to be species dependent. The most abundant compound in red leaf lettuce was cichoric acid (a dicaffeoyltartaric acid) while rosmaric acid (an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid) dominated in basil. Other compounds detected also varied between the species. Red leaf lettuce was much more responsive to supplemental blue light. Based on these results, it is suggested that both blue and red light may be needed to regulate the accumulation of phenolics in basil. Some of the compounds detected accumulated continuously as a function of the spent time under supplemental blue light in red leaf lettuce, but not in basil.

1. Introduction

Optimal plant growth requires both blue and red ranges of the light spectrum, because the two absorption peaks of chlorophyll molecules occur at these wavelengths. Thus commercially available greenhouse lights, which make use of new LED techniques, build their spectrum accordingly. However, there are also other light-driven processes in plants. Photomorphogenesis modifies a plant's form and shape using specific parts of the light spectrum (Cashmore, 2006) and specific wavelengths may enhance production of certain phytochemicals in plants (e.g., Li and Kubota, 2009).

Precise allocation of limited resources to increase competitive ability and defense is critical for a plant's survival (e.g., Ballaré, 2014). Spectral light composition may provoke "trade-off" between optimal growth and defense systems. Enhanced blue light (400–500 nm), for example, may strongly increase the biosynthesis of phenolic compounds (Taulavuori et al., 2013) as well as epidermal flavonoids (Hoffmann et al., 2014). The essential oil content of basil plants grown under blue light has been found to be between 1 and 4 times higher than those grown without blue light (Amaki et al., 2011). Phenolic compounds are an essential part of a plant's defense system (e.g., Bennet and Wallsgrove, 1994). On the other hand, blue light is a factor limiting growth (Taulavuori et al., 2005; Sarala et al., 2007, 2011). Relatively high quanta of red light (around 660 nm), or a high red to far-red ratio, act principally in similar ways—suppressing growth and stimulating the defense system via jasmonic acid (JA) biosynthesis. Effects of blue and red light obviously share some mechanisms, since shady environments with low levels of these lights cause shade-avoidance syndrome through increasing activity of phytochrome-interacting factors (PIFs) (Ballaré, 2014, and references therein).

It is known that biosynthesis of certain phytochemicals is stimulated by exposure to a specific light spectrum, although the overall mechanism is not well understood. Most of the thousands of phytochemicals have been found to be genus- or species-specific (e.g., Julkunen-Tiitto, 1989; Crozier et al., 1997; Pichersky et al.,

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2006; Cheynier et al., 2013). The specific question here concerns temporal changes during the life span of a plant: how soon is the seedling responsive enough to produce phytochemicals under light manipulation? Basil, for example, is able to accumulate anthocyanins in its early stages (8 days) of development, and the concentrations are highest prior to flowering (Phippen and Simon, 1998). Juvenile leaves in high-light environments commonly appear red as a result of anthocyanin accumulation, produced due to their photo-protective role (Hughes et al., 2007). While many phytochemicals are accumulated after growth has finished, many of them are also accumulated in large amounts during the juvenile stages (e.g., Bar-Peled et al., 1993, and references therein; Julkunen-Tiitto et al., 1996; Laitinen et al., 2002).

Son and Oh (2013) proposed that a mixture of blue and red lights enhance quality and yield in lettuce. They tested several blue to red ratios, and found the best yield with a red-weighted spectrum, and highest production of total phenols and total flavonoids by a blue-shifted spectrum. The aim of our work was to experimentally compare the effects of blue- and red-weighted spectra on two species, and to examine responses in phytochemical production when extending the period for supplemental blue light. Hence, plants were exposed to a variable number of days of supplemental blue light. The species studied were edible herbs that exhibit different growth forms and are commonly used as food (i.e., red leaf lettuce and basil). One treatment received supplemental blue light throughout the experiment (48 days). In the other treatments, the plants received first red-weighted light for a differing number of days, because the red is thought to be optimal for growth under LED exposure (Son and Oh, 2013). After each 12 days period, one treatment was changed to receive supplemental blue light, i.e., after 12, 24 and 36 days. One treatment remained as a red-weighted control throughout the experiment. Thus the supplemental blue light periods were 0, 12, 24, 36 and 48 days, in order to investigate temporal changes in phytochemical accumulation. It was hypothesized that (1) the possible responses are species-specific, (2) and blue light rather than red is behind the phenolic acid and flavonoid biosynthesis. It was also hypothesized that (3) certain flavonoids accumulate as a function of time, (4) while the developmental stage of the plant may affect the response (juvenile vs. older plants).

2. Materials and methods

The experiment was performed in the greenhouse of the Botanical Gardens, University of Oulu, Northern Finland (65°N). The two species studied were red leaf lettuce (*Lactuca sativa var* Lollo Rossa) and basil (*Ocimum basilicum* Cv genovese gigante). Lettuce with a rosette-like growth form (*Lactuca* sp.) has a relative low-flavor, but is widely used as a basic component of garden salads. Basil (*Ocimum* sp.) in turn is a culinary herb with leafy, erect stems. The red leaf lettuce was chosen for the study instead of green leaf lettuce due to its natural capability to produce anthocyanins, which is indicative of significant flavonoid biosynthesis. Our preliminary comparison between red and green cultivars (Lollo Rossa vs. Iceberg, respectively) confirmed this (unpublished). There are also other studies indicating that red leaf lettuce is more productive in phenolic compounds (e.g., Neocleous et al., 2014). Basil was chosen for the studied species as it is also known to be rich in phenolic compounds (Lee, 2010, and references therein).

The seeds were sown in 9×9 cm pots (0.75 l), filled with commercially available fertilized peat substrate (Berner Greencare, NPK 12-6-14, conductivity 20 m Sm^{-2}), on the 18th October 2012. Germination was carried out at +20 °C/RH 80% with a 9 h daily dim light period. The pots (6 of each species) were inserted into 25×35 cm boxes. There were 5 treatments levels and each treatment consisted of 4 replicate boxes ($n = 4$).

The experiment began on 2nd November and continued for 48 days as in our previous experiment, during which the outdoor light conditions (photoperiod, light intensity) were minimal, and decreased markedly over the experimental time (Taulavuori et al., 2013). The growth conditions in the greenhouse were as follows: temperature +18 °C, RH 60% and light intensity 300 μmol m^{-2} s^{-1} (sodium Philips Master Son-T Pia Green Power 400 W, high pressure sodium lamps) with 16 h photoperiod. The plants were watered at 3 day intervals with tap water and at the same time each rectangle shaped plot below the lamp system was rotated clockwise to equalize any light scattering effects. Thus the full rotation of the plots continued for 12 days, which was chosen as the temporal treatment unit of the experiment. Since the experiment continued for the 48 days, there were 5 treatments—exposure of 0, 12, 24, 36 and 48 days to enhanced blue light.

The specific light spectra were given by 120 W LED unit interlighting systems placed between traditional high pressure sodium (HPS) lamps (400 W) and plants, serving thus as supplemental light sources modifying the light spectrum. The LED units were around 60 cm above the plants and the HPS lamps were approximately 150 cm above the plants. Commercially available LED unit systems were used (Led Finland) to generate red-weighted growth light for optimal growth conditions (e.g., Son and Oh, 2013). The LED composition included 720 nm (1.8%), 660 nm (50%), 630 nm (30%), 450 nm (11%), 430 nm (3.6%), 410 nm (0.9%) and 3500 K white (2.7%). The specific, enhanced blue light spectrum boosted the range at 400–500 nm, which is described in detail elsewhere (Taulavuori et al., 2013), and was given as supplemental light to the HPS, in the same way as the red-weighted light. The spectra of both systems are shown in Fig. 1. At the beginning of the experiment, four treatments consisting of four replicate boxes ($n = 4$) received the red-weighted light (RW), and the fifth treatment received the enhanced blue light (EB). After each 12 days temporal unit, there was a switch in one treatment plot from red to blue: the RW LED system was replaced with the EB. Therefore, the experiment consisted of the following five treatments after 48 experimental days: 48, 36, 24, 12 and 0 days under enhanced blue light (see Fig 2. for the experimental design).

The experiment was terminated on the 20th December. Chlorophyll fluorescence ratio (Fv/FM) was first measured with a portable chlorophyll fluorometer (Walz PAM 2000) from 4 randomly chosen dark-adapted individuals of both species, per replicate box (Taulavuori et al., 2011). The 4 measurements were averaged for the value of one replicate in each treatment ($n = 4$). The shoot length of each basil plant was also measured with a ruler

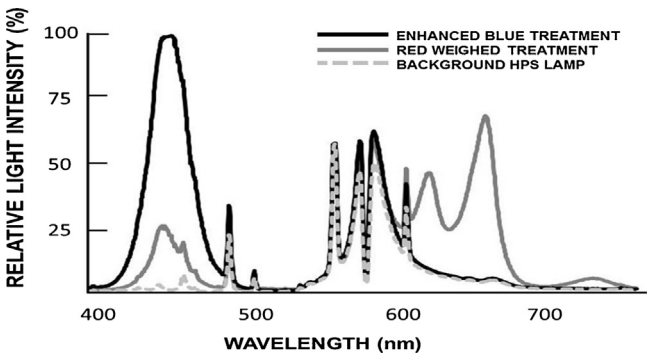

Fig. 1. Spectral compositions in EB (enhanced blue) and RW (red weighted) treatments, and background HPS (high pressure sodium) lamp (1 m above treatment lamps).

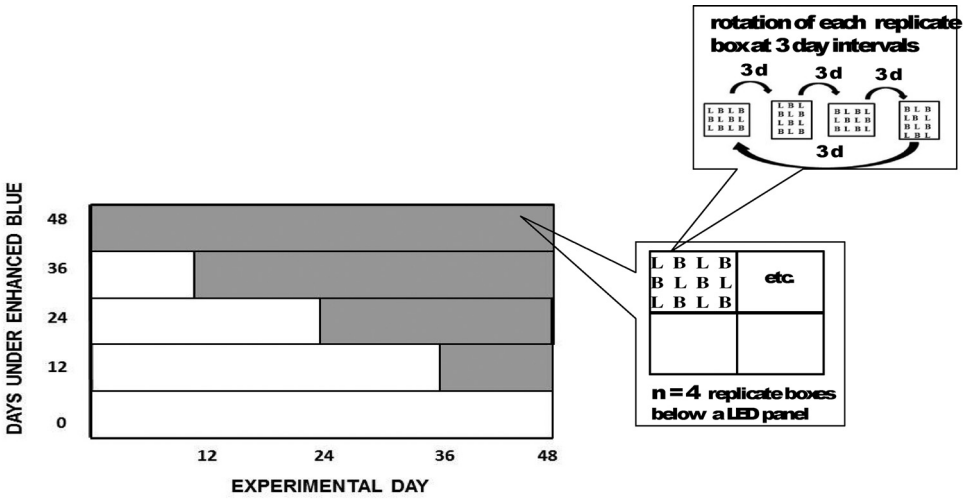

Fig. 2. Experimental design: there were 5 LED panels, one above each treatment, and their light beams overlapped the 4 replicate boxes (=*n*) inside the treatment. Six individuals of both species existed within each replicate. White horizontal bar indicates period of red weighted light exposure. Grey horizontal bar indicates period of enhanced blue light exposure. Rotation of the boxes side by side was performed at 3 day intervals. L = lettuce; B = basil.

to 1 mm accuracy, these were averaged for the replicate value. All the above-soil biomass was collected and air-dried at room temperature in a drying room (at relative humidity = 10%). The milled plant powder (8–10 mg) was extracted with 600 μl cold methanol for 30 s using a Precellys homogenizer (Bertin technologies, France), and left to stand on an ice bath for 15 min. The samples were centrifuged at 16,000 g for 3 min, and the supernatant was collected. The extraction was repeated four times (when the sample was left to stand on an ice bath for 5 min only). The supernatants were combined, and methanol was evaporated under gaseous nitrogen. The dried extracts were dissolved in 300 μl methanol and 300 μl Milli-Q-water. The samples were analysed by means of high performance liquid chromatography (HPLC, Agilent Technologies 1100/1200) (Julkunen-Tiitto and Sorsa, 2001). The compounds were quantified at either 220, 270 or 320 nm using commercial or purified standards. Identification of the compounds was carried out as described in Taulavuori et al. (2013). The compounds identified are summarized in Table 1.

Statistical comparisons between the treatments ware performed with one-way ANOVA (IBM SPSS Statistics 20 Software). A simple permutation test gave identical results with parametric ANOVA. The post hoc comparisons were made using Scheffé's test, and it the cases of unequal variances, the Games–Howell comparison was used.

3. Results

Ten bioactive phenolic acids or flavonoids were detected in red leaf lettuce during the experiment. Six compounds out of ten changed in concentration significantly under blue light enhancement (Table 2). Concentration of protocatechuic acid increased during the first 36 days ($P < 0.05$), but dropped then to the level of plants under only the red-weighted treatment (i.e., zero days under enhanced blue). There were no spectral responses in chlorogenic acid, chlorogenic acid derivative and quercetin-diglucoside concentrations. Quercetin-malonyldiglucoside concentrations were highest in plants that experienced the longest period of enhanced blue light ($P < 0.05$). Quercetin 3-malonyl-glucoside increased as a function of extending the period under enhanced blue light ($P < 0.001$). A similar response occurred in concentrations of cichoric acid ($P < 0.001$). Cichoric acid derivative remained stable for 24 days, but increased finally when plants had experienced enhanced blue light for 36 and 48 days ($P < 0.01$). The response in quercetin rhamnoside was quite similar ($P < 0.01$). Apigenin derivative was the only compound that displayed a negative trend, although not statistically significant, for blue light treatment.

Eight bioactive compounds in addition to two amino acids were detected in the chromatograms of basil, where blue light

Table 1
Identification of the compounds induced by the treatment using UV-spectrum and LC Q-TOF mass spectrometry.

Compound	LC-retention time (min)	Plant species	Identification method		
		a = lettuce; b = basil	UV-spectrum/MS	Q-TOF (pos.)	Q-TOF (neg.)
Protocatechuic acid	1.54	a	UV-spectrum		
Gallotannin derivative	7.89	b	UV-spectrum		
Chlorogenic acid	9.84	a,b	UV-spectrum/MS	377,0847 (M + 23)	353,0966 (M − 1)
Chlorogenic acid derivative	10.38	a	UV-spectrum		
p-OH-cinnamic acid derivative	11.65	b	UV-spectrum		
Quercetin diglucoside	12.32	a	UV-spectrum/MS	627,1555 (M + 1)	
Quercetin-malonydilglucoside	14.95	a	UV-spectrum		
Quercetin-malonylglucoside	18.98	a	UV-spectrum	551,1038 (M + 1)	
Chicoric acid	21.21	a,b	UV-spectrum/MS	497,0696 (M + 23)	473,0727 (M − 1)
Chicoric acid derivative	21.92	a	UV-spectrum		
Quercitrin	22.72	a,b	UV-spectrum/MS	471,0908 (M + 23)	
2-O-feruloyl tartaric acid	23.7	b	UV-spectrum/MS	349,1264 (M + 23)	
Rosmarinic acid	24.28	b	UV-spectrum/MS	383,0743 (M + 23)	359,0781 M − 1)
Lignan derivative	25.17	b	UV-spectrum		
Apigenin derivative	26.25	a	UV-spectrum		

Table 2
Bioactive compounds in lettuce that increased under enhanced blue light in comparison to plants under a red- weighted spectrum. Sig. indicates ANOVA differences between the days under enhanced blue. The different letters (a, b, c) denote for the differences in Scheffé's post hoc comparison, except for those when significance was highlighted with as asterisk to indicate heterogeneity of variances. Games–Howell comparisons were used in these cases. The number of replicates is four ($n = 4$), except for days 0 and 12 ($n = 3$). The values indicate mean concentrations (mg g^{-1}, dw) ± SE.

Compound	Days under enhanced blue					
	0	12	24	36	48	Sig.
Protocatechuic acid	0.012 ± 0.001 a	0.013 ± 0.001 ab	0.014 ± 0.001 b	0.016 ± 0.001 c	0.012 ± 0.003 abc	$P < 0.05$ *
Chlorogenic acid	11.486 ± 0.396	11.962 ± 0.409	11.411 ± 0.653	11.732 ± 0.285	11.953 ± 0.494	NS
Chlorogenic acid derivative	0.084 ± 0.010	0.076 ± 0.002	0.087 ± 0.001	0.078 ± 0.013	0.088 ± 0.003	NS
Quercetin diglucoside	0.072 ± 0.004	0.081 ± 0.004	0.084 ± 0.004	0.080 ± 0.007	0.076 ± 0.008	NS
Quercetin-malonyl diglucoside	0.216 ± 0.018 a	0.238 ± 0.007 a	0.237 ± 0.030 ab	0.223 ± 0.020 a	0.273 ± 0.014 b	$P < 0.05$ **
Quercetin 3-malonylglucoside	0.132 ± 0.009 a	0.152 ± 0.005 a	0.185 ± 0.031 ab	0.206 ± 0.013 b	0.219 ± 0.014 b	$P < 0.001$ *
Cichoric acid	21.478 ± 0.231 a	23.129 ± 0.861 ab	24.121 ± 2.096 ab	25.781 ± 0.514 b	27.512 ± 0.476 c	$P < 0.001$
Cichoric acid derivative	3.903 ± 0.224 ab	3.919 ± 0.399 ab	3.887 ± 0.477 a	4.754 ± 0.236 b	4.739 ± 0.276 b	$P < 0.01$
Quercetin rhamnoside	2.931 ± 0.319 a	3.409 ± 0.237 ab	3.594 ± 0.493 ab	4.139 ± 0.203 b	4.115 ± 0.437 b	$P < 0.01$
Apigenin derivative	0.127 ± 0.001	0.014 ± 0.001	0.014 ± 0.003	0.014 ± 0.002	0.015 ± 0.002	NS

* Games–Howell post hoc.

stimulated production of only two compounds (Table 3). Cichoric acid and quercetin rhamnoside increased during the experiment ($P < 0.05$). Many compounds showed a tendency, while non-significant, to decrease from the second longest exposure (36 days) to the longest exposure (48 day) of enhanced blue.

Measurements on chlorophyll fluorescence ratio (Fv/Fm) indicate that red leaf lettuce experienced no stress during the experiment (Fig. 3). The ratio was generally slightly lower in basil, although the difference was not statistically significant. Shoot elongation of basil tended to decrease, while not significantly, as a function of increased days under enhanced blue (Fig. 4).

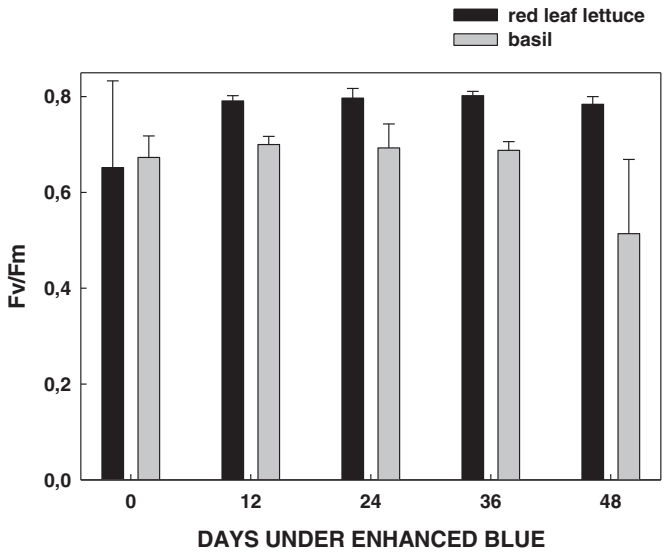

Fig. 3. Chlorophyll fluorescence ratio of lettuce and basil at the end of experiment ($n = 4$; error bar SE).

4. Discussion

Cichoric acid and rosmaric acid exist naturally as major phenolic compounds in lettuce (Rajashekar et al., 2012; Becker et al., 2013) and basil (Lee and Scagel, 2009; Taulavuori et al., 2013), respectively. In accordance with previous studies, cichoric acid was the most abundant phenolic compound found in red leaf lettuce (Table 2) and similarly rosmaric acid in basil (Table 3). In accordance with our previous study (Taulavuori et al., 2013), the enhanced blue light increased only few of the flavonoid compounds in basil. This may indicate that the phenolic pool is not the most responsive to changes in light quality in this plant species. This is supported by the fact that terpenoid biosynthesis may be highly increased under environmental stress in basil (Misra et al., 2014). And indeed, the blue light has induced essential oil production in basil, especially under blue light exposure (Amaki et al., 2011). However, the supplemental blue light has increased rosmaric acid concentration up to 4 fold compared to plants grown under high sodium pressure lamps (Taulavuori et al., 2013). In the present work, the rosmaric acid in basil was relatively high in all treatments, but marginally decreased during the experiment in response to the extension of the blue light period.

It has been shown that specifically red light increases the rosmaric acid concentration in basil (Shiga et al., 2009). This indicates that rosmaric acid biosynthesis is reactive to both blue and red wavelengths. However, cichoric acid production was boosted clearly by supplemental blue light in both species (Tables 2 and 3). When compared to our previous study (Taulavuori et al., 2013) analogous findings may be observed in concentrations

Table 3
Bioactive compounds in basil that increased under enhanced blue light in comparison to plants under a red- weighted spectrum: the explanations as in Tab. 2. The number of replicates is four ($n = 4$).

Compound	Days under enhanced blue					
	0	12	24	36	48	Sig.
Gallotannin derivative	0.015 ± 0.005	0.015 ± 0.005	0.019 ± 0.003	0.018 ± 0.005	0.017 ± 0.003	NS
Chlorogenic acid	0.889 ± 0.040	1.022 ± 0.185	1.205 ± 0.158	1.180 ± 0.243	1.211 ± 0.126	NS
p-OH cinnamic acid derivative	0.266 ± 0.025	0.318 ± 0.077	0.331 ± 0.053	0.399 ± 0.110	0.339 ± 0.088	NS
Cichoric acid	6.649 ± 0.544	5.812 ± 0.674	7.783 ± 1.156	7.943 ± 0.877	7.810 ± 1.081	$P < 0.05$
Quercetin rhamnoside	0.486 ± 0.049	0.592 ± 0.144	0.744 ± 0.096	0.719 ± 0.065	0.750 ± 0.160	$P < 0.05$
Feruloyl tartaric acid	0.437 ± 0.058	0.418 ± 0.024	0.488 ± 0.069	0.480 ± 0.018	0.488 ± 0.067	NS
Rosmaric acid	18.034 ± 3.498	17.424 ± 1.340	18.048 ± 4.190	18.549 ± 2.143	15.897 ± 1.068	NS
Lignan derivative	1.023 ± 0.153	1.166 ± 0.229	1.146 ± 0.378	1.328 ± 0.210	0.748 ± 0.229	NS

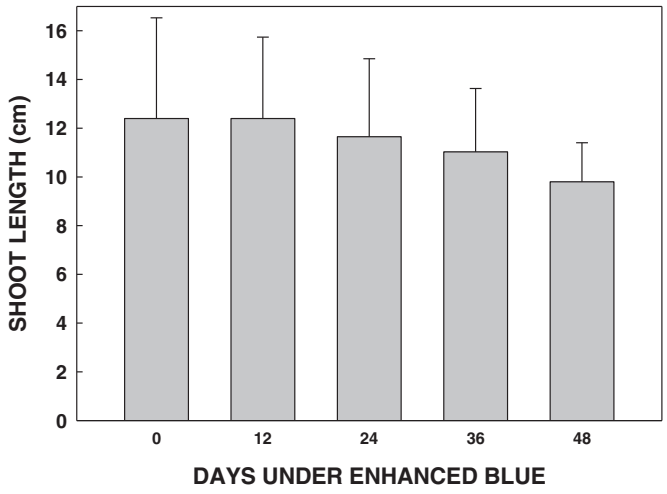

Fig. 4. Shoot elongation in basil in different treatments during the experiment ($n = 4$; error bar SE).

of chlorogenic acid, *p*-OH cinnamic acid derivative and feruloyl-tartaric acid in basil. There were no differences in concentrations of these compounds between blue and red light treatments in this study (Table 3), while supplemental blue increased these compounds significantly compared to basil plants grown under high sodium pressure lamps (Taulavuori et al., 2013). This indicates that biosynthetic accumulation of these phenolic acids may be increased both blue- and red- weighted spectra, similarly rosmaric acid in basil.

The red leaf lettuce is known to be rich in quercetins and chlorogenic acid (Crozier et al., 2000; Arabbi et al., 2004; Becker et al., 2013). Total phenols, chlorogenic acid and anthocyanin concentrations in the seedlings of this cultivar were found to be significantly higher under blue-containing LEDs (Johkan et al., 2010). There may exist a direct link between blue light biosynthesis of certain phenylpropanoids through photosynthesis. Becker et al. (2013) showed a close correlation between quercetin-glycosides concentrations with the reducing sugar concentration indicating that glucose might directly increase glucosylation of certain flavonoid aglycones to their glucosides. Accordingly, concentrations of two quercetin-glucosides increased in red leaf lettuce under supplemental blue light (Table 2).

The two species investigated responded differentially in terms of phytochemical accumulation, in accordance with the first hypothesis and previous literature (e.g., Julkunen-Tiitto, 1989; Crozier et al., 1997; Pichersky et al., 2006; Cheynier et al., 2013). Taken together, supplemental blue light increased many bioactive compounds in red leaf lettuce, while in basil only two compounds show this response. In addition, although cichoric acid increased under supplemental blue light in basil, its concentration was also relatively high under the red-weighted light. Finally, lettuce and basil yielded a different mixture of substances. Thus, these findings support the hypothesis that possible responses are species-specific. Consequently, the hypothesis that blue light rather than red is behind the phenolic acid and flavonoid biosynthesis, is only partially supported. The species-specific difference was evident in relation to hypothesis 3: certain flavonoids accumulated over time in red leaf lettuce while not in basil. However, hypothesis 4 – according to which the developmental stage may affect the response – can be neither supported nor rejected by the data.

In addition to the light spectrum, the species-specific responses may also be modified by other aspects of light. For example, light intensity may be a factor behind the regulation of these responses. Especially dihydroxy-B ring-substituted flavonoids (e.g., quercetin glycosides) have been found to be up-regulated by high irradiance (Agati et al., 2013). As the light saturation level of basil is much higher, exceeding >1000 $\mu mol\ m^{-2}\ s^{-1}$, compared to the respective level (<300–400 $\mu mol\ m^{-2}\ s^{-1}$) of lettuce (e.g., Kitaya et al., 1998; Chang et al., 2008), it is understandable that basil was not as responsive to the experimental treatments as lettuce under the light intensity supplied (300 $\mu mol\ m^{-2}\ s^{-1}$). It should be remembered that red leaf lettuce is bred for production of high anthocyanins, the pigments causing the red color, and giving further protection against high light intensity.

There were practically no differences in shoot elongation of basil grown under either red-weighted or blue dominating light (Fig. 4). This result is in accordance with the growth suppressing effect of red light found by (Ballarě, 2014, and references therein), and the conclusion that removal of blue light increased elongation of many species (Sarala et al., 2011). In Scots pine seedlings the observed stem elongation is thought to be a photomorphogenic response, which is not dependent on gas exchange or accumulation of growth resources (Sarala et al., 2009). These reactions may be explained by flavonoids through altered *p*-coumaroyl CoA metabolism mediating photomorphogenic responses (e.g., Briggs and Huala, 1999; Folta and Spalding, 2001; Parks et al., 2001). Flavonoids and monolignols are synthesized by two different routes: if synthesis of quercetin and other flavonoids is enhanced under blue light it may lead to a decrease in the monolignols needed in lignin biosynthesis. This may cause decreased growth through affecting chalcone synthase activity and auxin transport. In fact, Besseau et al. (2007) showed that repression of lignin synthesis in *Arabidopsis thaliana* leads to the light-controlled redirection of the metabolic flux into flavonoids and they concluded that the reduced size phenotype of the plants is due to the presence of flavonoids. Moreover, inhibition of the condensed tannin pathway leads to the accumulation of the compounds preceding the phenylpropanoid pathways (Kosonen et al., 2015). The increase is especially strong in flavonol derivatives, for example quercetins which are known to be among the most active phenolic compounds in perturbing auxin transport. Moreover, flavonoids have been shown to be located in the nucleus where they may control the transcription of genes involved in growth and development (e.g., Saslowsky et al., 2005).

For future prospects, it is interesting to identify in more details, which range or peak of the blue light is inducible for the biosynthesis of a given specific flavonoid or phenolic acid. Indeed, the question concerns the UV radiation as well, since both UV-A (e.g., Wilson et al., 2001) and UV-B radiation (e.g., Gerhardt et al., 2008) modify flavonoid composition. The enzyme behind flavonoid biosynthesis is chalcone synthase (CHS), and it is shown that UV-A radiation and blue light inductions in CHS expression are mediated by cryptochrome (cry1) (Wade et al., 2001). Phenylalanine, a precursor in flavonoid biosynthesis, is required for specific developmental responses mediated by UV radiation (Sullivan et al., 2014). It is also well-known, that UV-B radiation limits growth and alters plant morphology (e.g., Teramura and Sullivan, 1994), i.e., the changes also responsive to blue light (e.g., Sarala et al., 2011).

In conclusion, this study supports earlier work, according to which the flavonoid and phenolic acid biosynthesis in plants are species (genetic) dependent. However, manipulation of the environmental light spectrum may boost the synthesis of certain products. Of the species studied, red leaf lettuce was much more responsive to supplemental blue light. Supplemental blue light increases production of these compounds especially in red leaf lettuce, while both blue and red light may regulate their production in basil. Some of the compounds detected accumulated continuously as a function of the time spent under supplemental blue light in red leaf lettuce, but not in basil.

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