Green light induces shade avoidance to alter plant morphology and increases biomass production in Ocimum basilicum L.

L. Schenkels [a*], W. Saeys [a], A. Lauwers [b] and M.P. De Proft [a]

[a] KU Leuven, Department of Biosystems, Willem de Croylaan 42, B-3001 Leuven, Belgium

[b] Vegobel, Missestraat 27, B-2570 Duffel, Belgium

[*] Corresponding author. Email address: liesbeth.schenkels@kuleuven.be

Scientia Horticulturae (2020) 261, 109002

https://doi.org/10.1016/j.scienta.2019.109002

Abstract

The consequence of adding green light (505nm) to a white light spectrum (350-700 nm) and the partial replacement of blue and red light by green light within a white light spectrum were evaluated for *Ocimum basilicum* L. cv. Marian regarding plant growth and development. Plants were grown in absence of far-red light consequently making green light the only light passing through the leaves. Green light (15 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was added to a white light spectrum (108 $\mu mol \cdot m^{-2} \cdot s^{-1}$) without changing the absolute intensity of the blue and red wavelengths. Adding green light to the spectrum did not affect the chlorophyll content in the leaves, nor the ratio of leaf length over leaf width. An increased biomass production, number of leaves per pot, number of leaves per stem, stem length, and individual leaf area were observed. These features are important quality attributes determining the marketable value of young basil. The partial replacement of blue and red light by green light resulted in a significant increase in biomass production compared to plants produced under a control spectrum at equal light intensity. A relative increase in green light also significantly increased stem length, leaf length and projected single leaf area. This study highlights the growth benefits of green wavelengths that are easily transmitted to the underlying leaves. We evaluated the effects of green light on basil morphology and its potential to induce shade avoidance symptoms. By observing increases in stem and leaf elongation, we have shown that green light could be used to alter the appearance and commercial value of basil.

Keywords

Basil (*Ocimum basilicum* L.), Green light, LED, Plant morphology, Shade avoidance, Light quality

1. Introduction

Plants perceive photoperiod, light quantity, and light quality as informative cues about the surrounding growth environment (Kami *et al.*, 2010; Zhang *et al.*, 2011). Photosynthetically active radiation (PAR) between 400 and 700 nm is important to generate biomass (McCree, 1971). The photoperiod is important in flower initiation and development in many blooming plants (Song *et al.*, 2013). Plants utilize light to detect the presence of neighbors, day time and seasons (Bantis *et al*., 2018).

Plant photoreceptors such as UV-B receptors, cryptochromes, and phytochromes have been known to detect UV, blue, and red light, respectively, but no specific green light receptor has been identified yet (Bantis *et al*., 2018; Folta, 2005; Macedo *et al*. 2011; Possart *et al*., 2014). While most studies have focused on the effects of red and blue light, a number of studies have focused on green light effects (Johkan *et al*., 2012; Kim *et al*., 2004; Olle and Viršile, 2013). Folta (2005) suggested that green light could be the antagonist to blue light responses. Macedo *et al*. (2011) reported that the effects of green light were in respect similar to those of red light, and in other ways similar to those of blue light. They argued that this can be explained by the fact that phytochrome and cryptochrome partially absorb green light (Macedo *et al*., 2011). Talbot *et al*. (2002) hypothesized that there could be a zeaxanthin-based compound acting as a green light

receptor (Folta and Maruhnich, 2007; Talbott *et al*., 2002). However, to date no dedicated light receptor has been identified specific for green light. Consequently, green light responses are not yet fully understood. Since the revolution in light research instigated by LED technology, the effects of green light on plant growth and development have been investigated by several research groups (Johkan *et al.*, 2012; Kaiser *et al.*, 2019; Kim *et al.*, 2004; Snowden *et al.*, 2016; Zhang *et al.*, 2011)

Green light was used as 'safe light' in the past (Mandoli and Briggs, 1981; Smith *et al*., 2017). It was assumed that green light would only contribute to photosynthesis to a minimal extent, and that it would have no effect on plant morphogenesis and function. However, more recent studies clearly present that plants are not blind to green light and that its role cannot simply be neglected (Folta, 2004; Folta and Maruhnich, 2007; Johkan *et al*., 2012; Kaiser *et al*., 2019; Kim *et al*., 2004; Macedo *et al*., 2011; Snowden *et al*., 2016; Terashima *et al*., 2009). For example, increasing the percentage of green light, often within a fixed Photosynthetic Photon Flux Density (PPFD), has been reported to increase the dry matter production of lettuce (*Lactuca sativa* 'Waldmann's Green'), tomato (*Solanum lycopersicum*, cv. Early girl), cucumber (*Cucumis sativa*, cv. Sweet Slice), pepper (*Capsicum annum*, cv. California Wonder), soybean (*Glycine max*, cv. Hoyt) and wheat (*Triticum aestivium* L. cv. USU-Apogee) (Kim *et al*., 2004; Snowden *et al*., 2016). However, no significant effect on dry weight was demonstrated for tomato (*Solanum lycopersicum* L.) by Arena *et al*. (2016) or in lettuce (*Lactuca sativa* var.crispa 'Green Oak Leaf') when including green light (Chen *et al*., 2016). Kaiser *et al*. (2019) demonstrated that the partial replacement of red and blue light by green light increased biomass, stem length and specific leaf area in tomato (*Solanum lycopersicon* L. 'Komeett'). Johkan *et al*.

(2012) reported that the effect of green light on leaf area and fresh weight for lettuce (*Lactuca sativa* L. cv Banchu Red Fire; Takii Seed Co., Kyoto, Japan) shifts when a different light intensity is applied. In *Arabidopsis thaliana*, green light induced shade avoidance symptoms in a background of red and blue light. Elongated petioles and upward leaf reorientation were observed (Zhang *et al*., 2011). Meng *et al*. (2019) reported that for lettuce and kale (*Brassica oleracea* var. sabellica) an increase in the relative proportion of green light in the spectrum by decreasing the amount of blue light resulted in shade avoidance symptoms (Meng *et al*., 2019).

While effects of green light on plant morphology have been reported, there is contradiction among these reports (Kaiser *et al*., 2019). This discrepancy might be explained by the use of different experimental setups. A common factor in many studies is that the total PPFD was kept constant over different treatments. This means that absolute intensities of blue and red wavelengths were reduced when green light intensity was increased. Blue and red wavelengths are known to have an important effect on plant morphology. When changing these absolute intensities, and ratios to other wavelengths, changes in plant morphology can be expected. Therefore, most of these studies did not allow discrimination between the growth effects due to the change in the blue and red wavelengths and those due to green light changes. To overcome this problem, the aim of this study was to gain insight in the effects of additional green light (505 nm) within a broad white light spectrum (350-700 nm) on plant growth and development. Plants were grown under white light containing additional green light without changing the absolute intensity of red and blue light. To be able to distinguish between the effects due to the additional green light and those due to the increased light intensity, an additional control

treatment was included with a light intensity equal to that of the green light enriched spectrum. This treatment allows to study the partial replacement of blue and red light by green light compared to additional green light.

Basil is a vegetative, compact plant with a short cultivation cycle (~28 days). It is one of the most important aromatic herbs worldwide with a high-added value making it an excellent study object for the application of new, often still expensive, lighting techniques (Akbari *et al*., 2019). Furthermore, it is an interesting model organism for other aromatic herbs and vegetative plants since it has clearly pronounced vegetative features. This study was executed in a far-red light excluded background making green light the only light passing through the leaves and the only wavelength possibly inducing a shift in plant morphology. This study evaluated the effects of green light on basil morphology and its potential to induce shade avoidance phenotypes. It is expected that increasing the relative amount of green light in the light spectrum will simulate the green light enriched light conditions in the lower levels of the canopy.

2. Material and methods

2.1 Plant material

Plant containers (8cm diameter) filled with a mix of peat, compost and perlite were used. Seeds (*Ocimum basilicum* L. cv. Marian, 30 seeds per plant container) were sown on top of the substrate and thereafter incubated in a germination room (21°C, ~100% RH) for 3 days before transfer to the specific location for the start of the experiment.

2.2 Growth conditions

Plants were kept in climate conditioned growth rooms (2 m², $T_{day\ (mean)}$ = 22.2 °C, $T_{night\ (mean)}$= 16.9 °C) during their whole cultivation period. Two multispectral LED lamps (max. 150 W each) were placed 35 cm above crop level. A 20-hour photoperiod was set from 4:00 am up to 00:00 am. The used nutrient solution had an EC value of 670 $\mu S.cm^{-1}$ (measured at 25 °C) and was kept at 22 °C. Plants were watered each morning (8:00 am) for 10 minutes by submerging them for 2 cm using an automated system. Spectral composition of the different light treatments and light transmission through the leaves was measured using a calibrated spectroradiometer at plant level (BLACK-CXR-SR-50, StellarNet Inc. Tampa, Florida, USA). In Figure 1, the spectral composition is visualized for the different light conditions used during these experiments. As a control treatment, a white light spectrum was used (108 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 'Control low intensity'). To construct the white light treatment a cool white LED (λ_{max}= 440 nm and λ_{max}= 570 nm) and two red LEDs (λ_{max}= 633 nm and λ_{max}= 664 nm) were used. A green light treatment (123 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 'Green high intensity') was constructed by adding green light (15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level, λ_{max}= 505 nm) to the 'Control low intensity' spectrum. In this way, the intensity of the green light increased without changing the absolute intensity of the blue and red wavelengths. To be able to separate the effects of the additional green light from the effects induced by the increased light intensity, a second control treatment was included by rescaling the 'Control low intensity' spectrum to match the light intensity obtained when adding green light (123 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 'Control high intensity'). Table 1 provides an overview of the light quantities used to construct the different light regimes.

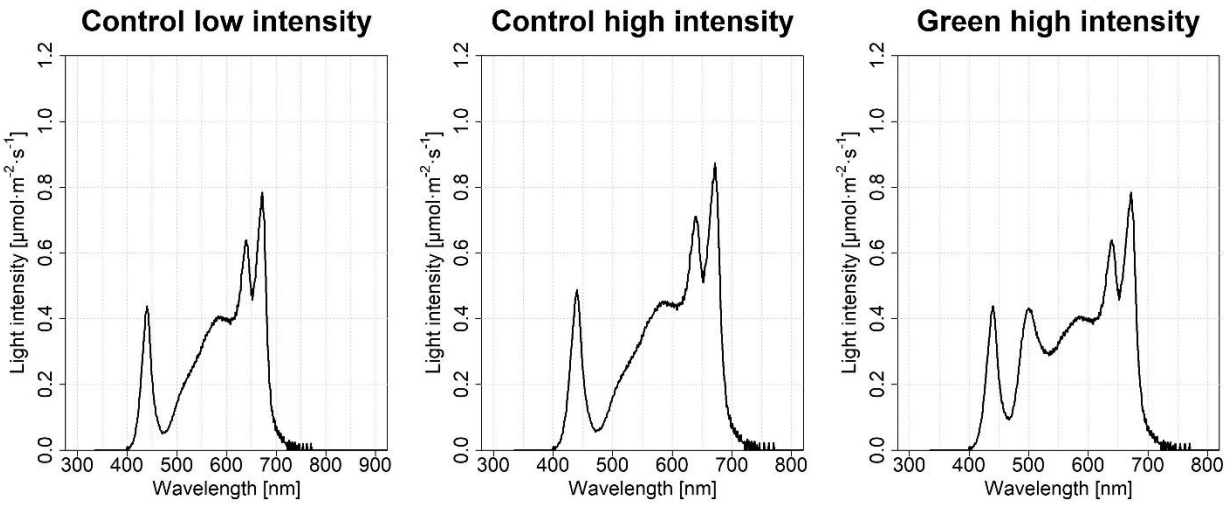

Figure 1: Spectral composition of the different light treatments applied: 'Control low intensity' (108 µmol·m^{-2}·s^{-1}), 'Control high intensity' (123 µmol·m^{-2}·s^{-1}), 'Green high intensity' (123 µmol·m^{-2}·s^{-1}).

Table 1: Light quantities and spectral characteristics used in the different light treatments applied: 'Control low intensity', 'Control high intensity', 'Green high intensity'. PPFD = Photosynthetic Photon Flux Density

Treatment	**Control low intensity**	**Control high intensity**	**Green high intensity**
Basic spectrum [µmol·m^{-2}·s^{-1}]	108	123	108
Additional green light (λ_{max} = 505 nm) [µmol·m^{-2}·s^{-1}]	0	0	15
PPFD [µmol·m^{-2}·s^{-1}]	108	123	123
Relative share of different wavelength bands [%]			
[400-450nm[	10.11	10.12	8.11
[450-500nm[	5.20	5.20	14.50
[500-550nm[	12.63	12.63	19.51
[550-600nm[	20.80	20.80	16.85
[600-650nm[	26.60	26.60	21.29
[650-700nm[	24.74	24.74	19.80

2.3 Plant biometrics

2.3.1 Chlorophyll content

The chlorophyll content of fully light-exposed leaves was determined using a non-destructive chlorophyll sensor (Dualex® Scientific, FORCE-A). A calibration curve for basil leaves was generated using a DMFA-extraction protocol (Wellburn, 1994) allowing the conversion of relative fluorescence values into absolute chlorophyll figures [mg Chl.g fresh leaf^{-1}].

2.3.2 Plant biometrics

At the end of the cultivation, plants from each treatment were scored. Full stem length [cm], leaf number (total per pot) and stem number (>5 cm, total per pot) were determined. The mean number of leaves per stem was calculated using the total number of leaves and stems per pot. For each pot the fresh weight [g], dry weight [g] and dry matter content [%] were determined for leaves and stems separately. The leaf-to-stem ratio was calculated by dividing the total fresh weight of leaves per pot by the total fresh weight of stems per pot. Dry weights were obtained after placing plant material in a drying oven (ICP500, Memmert GmbH + Co. KG, Germany) for 7 days at 70°C. The average weight of one single, fully grown leaf was calculated by weighing six leaves separately and dividing the obtained mass by six. The leaf length [cm] and leaf width [cm] of a fully expanded leaf (first leaf above the cotyledons) were determined using ‘ImageJ’ (version 1.52a, developed by Wayne Rasband, NIH, USA). The first leaf above the cotyledons was chosen, because it was a fully grown, true leaf. Evaluating the lower leaves allowed to compare leaves from a similar growth stage in the different light treatments. The

projected area of individual, fully grown leaves [cm^2] (first leaf above the cotyledons) was determined by k-means color clustering using the program ‘Image color summarizer’ (version 0.76, developed by Martin Krzywinski). Specific leaf area (SLA) [$cm^2.g^{-1}$] was calculated by dividing the area of an individual leaf by its mass.

2.4 Statistical analysis

‘Rstudio’ (version 3.2.5) was used for the statistical analysis and to generate the graphical representation of the obtained data. The Shapiro-Wilk test (‘shapiro.test’) was used to test the normal distribution of the data. If the assumption of normality was met, the comparison of the different treatments was performed using Tukey Multiple comparisons test (‘HSD.test’). If the assumption of normality was not met, and no suitable transformation could be found, the non-parametric Dunn test was used (‘Dunntest’).

3. Results

Figure 2 shows representative plants at the end of the cultivation period (29 days). Plants grown under additional green light appeared taller and bore a larger canopy.

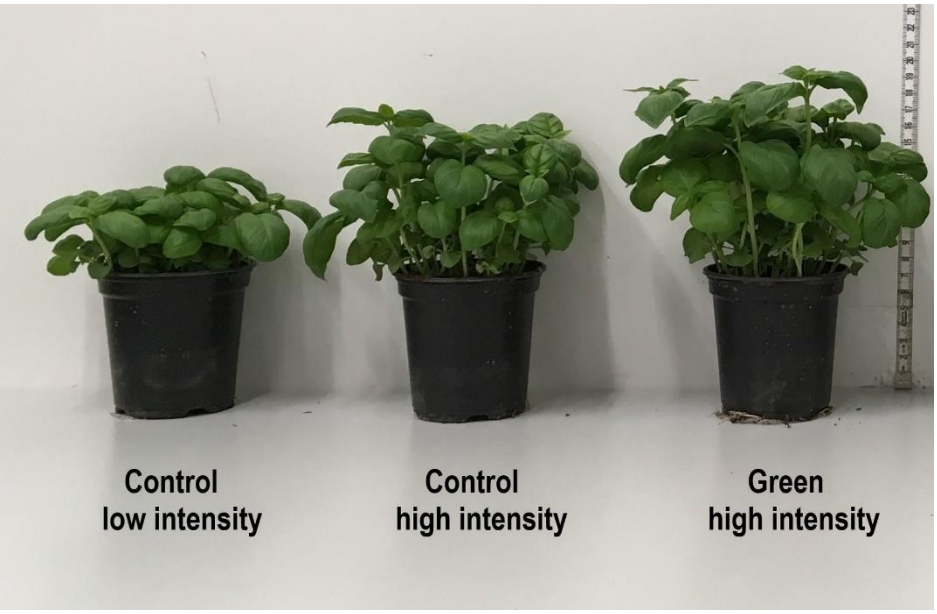

Figure 2: Ocimum basilicum L. cv. Marian at the end of the 29-day growing period. Representative plants from the 'Control low intensity', 'Control high intensity' and 'Green high intensity' are presented.

3.1 Effect of light recipes on light transmission through the canopy

The difference between the spectrum recorded on top of the canopy and after passing through a single leaf allows to calculate leaf transmission (Figure 3). Light transmission was found to be higher in the green-yellow (± 15% transmission) and far-red regions of the spectrum compared to the red and blue region (0% transmission) (Figure 3). At the green peak wavelength of 505 nm, leaves transmitted 5.5 % of the light applied. Maximal leaf transmission in the PAR region was recorded at 555 nm (18% transmission).

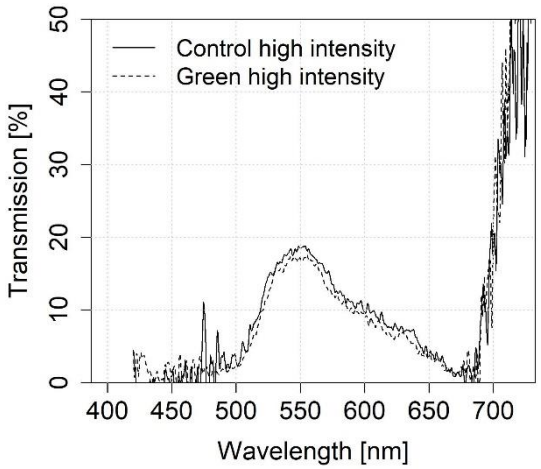

Figure 3: Light transmission by a single, full grown leaf (O. basilicum L. cv. Marian) at the end of the cultivation period from a 'Control high intensity' and 'Green high intensity' grown plant. Each spectrum is the average of 5 readings.

3.2 Effect of different light recipes on biomass production

The biomass distribution over the various plant parts for the different light treatments is presented in Table 2. By increasing the light intensity at plant level by 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ all biomasses of different plant parts significantly increased, except for the individual leaf mass. Total fresh weight increased by 63%, while dry weight increased by 68%. Dry matter content of the leaves increased when increasing light intensity, while the dry matter content of the stems was significantly reduced. Introducing additional green light ('Green high intensity') resulted in a significant increase of the total fresh and dry weight compared to the treatment with lower light intensity, but also to the control treatment with equal light intensity (Table 2).

Table 2: Biomass distribution of O. basilicum L. cv. Marian after 29 days of cultivation (± se) (n=10). Different treatments were compared: 'Control low intensity', 'Control high intensity' and 'Green high intensity' using Tukey HSD test (α=5%). Different letters between brackets indicate significant differences between the treatments.

Treatment	**Control low intensity**	**Control high intensity**	**Green high intensity**
Individual leaf mass [g]	0.27 ± 0.015 (B)	0.32 ± 0.012 (AB)	0.35 ± 0.010 (A)
Fresh weight leaves [g]	8.04 ± 0.38 (C)	12.45 ± 0.17 (B)	13.73 ± 0.33 (A)
Dry weight leaves [g]	0.75 ± 0.041 (B)	1.31 ± 0.017 (A)	1.43 ± 0.047 (A)
Dry matter content leaves [%]	9.39 ± 0.25 (B)	10.49 ± 0.15 (A)	10.39 ± 0.20 (A)
Fresh weight stems [g]	3.35 ± 0.095 (C)	6.12 ± 0.144 (B)	7.29 ± 0.171(A)
Dry weight stems [g]	0.23 ± 0.010 (C)	0.36 ± 0.013 (B)	0.43 ± 0.024 (A)
Dry matter content stems [%]	6.97 ± 0.31 (A)	5.93 ± 0.12 (B)	5.89 ± 0.25 (B)
Fresh total biomass [g]	11.39 ± 0.44 (C)	18.58 ± 0.28 (B)	21.03 ± 0.47 (A)
Dry total biomass [g]	0.99 ± 0.049 (C)	1.67 ± 0.027 (B)	1.86 ± 0.066 (A)
Dry matter content total [%]	8.65 ± 0.23 (A)	8.99 ± 0.12 (A)	8.83 ± 0.16 (A)

3.3 Effect of different light recipes on plant morphology

After 29 days of cultivation, stem length (n=30, 3 plants per pot), chlorophyll content of the upper leaves (n=40, 4 leaves per pot), number of leaves per pot (n=10) and number of stems per pot (n=10) were measured. The length and width of individual leaves and the projected single leaf area were determined for 20 leaves from each treatment (Table 3). Increasing the light intensity by 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, without changing the composition of the spectrum, resulted in a significant increase in stem length, leaf number and number of stems per pot. The leaf number per stem increased by 17%, while the leaf-to-stem ratio decreased by 15% when increasing the light intensity. All this resulted in plants with an

increased market value. When comparing the light conditions with equal light intensity ('Control high intensity' and 'Green high intensity'), more green light resulted in an increased leaf length, leaf width and projected single leaf area compared to a light spectrum containing relatively less green light. Stem length increased significantly when increasing the relative amount of green light (Table 3).

Table 3: Plant morphology of O. basilicum L. cv. Marian after 29 days of cultivation (± se). Different treatments were compared: 'Control low intensity', 'Control high intensity' and 'Green high intensity' using Tukey HSD test or Dunn test (*) (α=5%). Different letters between brackets indicate significant differences between the treatments.

Treatment	**Control low intensity**	**Control high intensity**	**Green high intensity**
Leaf length [cm]*	3.41 ± 0.057 (B)	3.38 ± 0.080 (B)	3.72 ± 0.057 (A)
Leaf width [cm]*	2.74 ± 0.037 (B)	2.79 ±0.056 (B)	3.10 ± 0.070 (A)
Ratio leaf length:leaf width	1.25 ± 0.030 (A)	1.22 ± 0.030 (A)	1.21 ± 0.026 (A)
Single projected leaf area [cm^2]	7.24 ± 0.10 (B)	7.31 ± 0.29 (B)	8.72 ± 0.28(A)
Specific leaf area (SLA) [$cm^2.\cdot g^{-1}$]	26.33 ± 0.37 (A)	23.19 ± 0.91 (B)	25.01 ± 0.81 (AB)
Stem length [cm]	4.88 ± 0.13 (C)	8.98 ± 0.20 (B)	10.90 ± 0.25 (A)
Chlorophyll content leaves [mg Chl.$\cdot g^{-1}$ fresh weight]	1.13 ± 0.034 (A)	1.17 ± 0.040 (A)	1.15 ± 0.045 (A)
Number of leaves per pot*	55.10 ± 1.94 (B)	75.60 ± 3.21(A)	78.60 ± 1.81(A)
Number of stems per pot*	19.00 ± 0.72 (B)	22.10 ± 1.02 (A)	22.00 ± 0.69 (AB)
Number of leaves per stem	2.92 ± 0.10 (B)	3.44 ± 0.077(A)	3.59 ± 0.082(A)
Leaf-to-stem ratio	2.40 ± 0.102 (A)	2.04 ± 0.040 (B)	1.89 ± 0.030 (B)

4. Discussion

4.1. Green light is transmitted through the canopy

It has been reported that most of the blue and red light is absorbed by a green canopy (80 - 100%) (Inada, 1976; Nishio, 2000; Terashima *et al*., 2009). This is confirmed by the results presented in this study (Figure 3). The transmission of blue and red wavelengths through a single basil leaf is very low due to the absorption peaks of chlorophyll in these blue and red regions (Smith *et al*., 2017). In the green light region, considerably higher transmission (5 - 15%) through the leaf was recorded (Figure 3). Increasing the amount of light in the region with relative higher transmission would be beneficial for the underlying leaves in the canopy, providing them with light to fuel photosynthesis. An increase in both biomass production and chlorophyll content of lower leaves has been linked with increased light transmission through the canopy (Hovi-Pekkanen and Tahvonen, 2008; Zhang *et al.*, 2015). Green light is known to penetrate deeper into the leaf compared to blue and red light (Sun, *et al.*, 1998). Not only the wavelength but also the direction at which the light is irradiated might affect light absorption in the leaf. When green light is emitted directly onto the leaf, light absorption could take place deeper into the leaf compared to low-angle or diffuse light (Brodersen and Vogelmann, 2010). Our study was deliberately conducted in the absence of far-red light. In nature, far-red light is transmitted through the upper leaves offering important light signaling cues to the underlying leaves. Increased levels of far-red light would result in increased stem elongation and other shade avoiding growth responses (Possart *et al*., 2014). Recently, green light has been linked to the induction of phenotypes related to the shade avoidance response in *Arabidopsis thaliana* (Zhang *et al.*, 2011). Zhang *et al*. (2011) quantified leaf

angle, petiole and leaf length in *Arabidopsis thaliana* and they concluded that a high relative amount of green light resulted in phenotypes similar to those observed in a shaded environment. This study on basil quantified the effect of enriched green light conditions, in the absence of far-red light, on other morphological traits such as stem length, chlorophyll content, and leaf dimensions that are easily impacted by shade avoidance syndrome.

4.2. Biomass production increased when increasing the relative amount of green light

When the total PPFD increased by 14%, the total fresh weight increased by 63% (Table 2). The light saturation point of *O. basilicum* has been shown around 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Beaman *et al.*, 2009). Therefore, the light intensities used in this study were situated in the linear part of the light response curve, explaining the significant increase in biomass production when increasing light intensity. Replacing red and blue wavelengths by green light ('Green high intensity') even further increased the biomass production by 13% compared to the reference spectrum of equal light intensity ('Control high intensity'). It has been reported that green light also contributes to photosynthesis, but in a less efficient way compared to blue or red light, with a relative quantum efficiency (RQE) of approximately 70% (McCree, 1971; Sager *et al.*, 1988). Therefore, a decrease in biomass production could have been expected for the plants grown under the same intensity, but with enriched green light. However, this was not observed in this study. McCree (1971) only determined the RQE for single leaf sections. When considering the canopy as a whole and associated green light transmission through the canopy, RQE might fall short to estimate total biomass production based on the RQE. This confirms the findings of

Kaiser *et al.* (2019) in tomato where the RQE was insufficient to estimate plant growth at canopy scale. The observed increase in biomass might be explained by the fact that the absorption of green light can take place deeper in the canopy, compared to blue and red wavelengths (Kim *et al*., 2004). In spinach leaves (*Spinacia oleracea* cv. Hybrid 424) it was shown that green light drives CO_2 fixation deeper into the leaf while blue and red light only reach the upper part of the leaf (Sun, *et al.*, 1998)

4.3. Stem length, leaf length and individual leaf area increased when increasing the relative amount of green light

When leaves are shaded by a green canopy, light intensity will decrease and the light spectrum reaching the lower leaves will become green and far-red enriched. Decreased chlorophyll levels in the lower leaves are linked to shade conditions and a reduced R:FR ratio (Smith and Whitelam, 1997). In this study, the chlorophyll content of the leaves was not significantly different between light treatments used (Table 3). Mickens *et al*. (2018) reported that there was no significant difference for the estimated chlorophyll content when including green light in the light spectrum when cultivating red romaine lettuce (*Lactuca sativa* cv. 'Outredgeous') (Mickens, et al., 2018). However, the inclusion of green light in the spectrum during pak choi (*Brassica rapa* var. chinensis, 'Rubi F1'), green butterhead lettuce (Lactuca sativa 'Rex'), red oakleaf lettuce (Lactuca sativa 'Rouxai') and kale (Brassica oleracea var. sabellica 'Siberian') cultivation resulted in a significant decrease in relative chlorophyll content (Meng, et al., 2019; Mickens, et al., 2019). Considering the absorption spectrum of chlorophyll, it is important to note that the absorption in the green light region is close to zero (Kang *et al*., 2018). Our study in basil showed that the relative amount of green light in the spectrum will not significantly alter

the chlorophyll content in the leaves. Stem length, leaf length and projected area of a single fully-grown leaf did increase significantly when increasing the relative amount of green light (Table 3). When plants experience shade, elongated stems and leaves can be expected due to the shade avoidance response (Smith and Whitelam, 1997). These findings further confirm the hypothesis of Zhang *et al*. (2011) that green light induces shade avoidance symptoms in the absence of far-red light. Kaiser *et al*. (2019) reported that in tomato stem length and specific leaf area significantly increased when increasing the relative amount of green light in a natural solar light background (including far-red light). Our study confirms these findings and adds that green light shade-avoidance signals can also be found in a non-far-red light background. The number of leaves and stems per pot increased significantly when increasing the light intensity but did not increase further when increasing the relative amount of green light. Johkan *et al.* (2012) reported that for lettuce the number of leaves did not increase when a white fluorescent lamp was replaced by different green LEDs at PPF 100 (photosynthetic photon flux) and PPF 200. Our study confirms that replacing red and blue wavelengths by green light did not significantly increase the number of leaves per pot or per stem.

Green light is a fairly abundant wavelength band in natural daylight (Smith, 1982). Furthermore, it is transmitted through the leaves, offering an excellent, highly stable light signal to deliver environmental information to the plant. Both green and far-red radiation trigger shade-avoidance responses in plants. We hypothesize that the presence of both green and far-red light would not augment the shade-avoidance response but increase the accuracy in which the plant can adapt its phenotype. The discovery of a green light photoreceptor would further help us in understanding this biological response.

5. Conclusion

Both additional green light and the partial replacement of the spectrum by green light resulted in an increased biomass production. This indicates that leaf-transmitted green wavelengths may be beneficial to the growth of the underlying leaf canopy. An increased stem length and leaf length were observed supporting the hypothesis that green light induces shade avoidance phenotypes. While the majority of the past light research has focused on blue and red light, this study indicates that green light should not be ignored when designing light regimes to improve plant production in commercial greenhouses and multilayer systems. An increased biomass production and overall plant quality can be achieved when fine-tuning the relative amount of green light.

Acknowledgements

The authors acknowledge the financial support of the Flemish ‘Agentschap Innoveren & Ondernemen’ [project number 150700 KUL/Vegobel/VLAIO].

References

Akbari, G. A., Binesh, S., Ramshini, H., Soltani, E., Amini, F., & Mirfazeli, M. S. (2019). Selection of basil (Ocimum basilicum L.) full-sib families from diverse landraces. *Journal of Applied Research on Medicinal and Aromatic Plants*, *12*, 66–72.

Bantis, F., Smirnakou, S., Ouzounis, T., Koukounaras, A., Ntagkas, N., & Radoglou, K. (2018). Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs). *Scientia Horticulturae*, *235*, 437–451.

Beaman, A. R., Gladon, R. J., & Schrader, J. A. (2009). Sweet Basil Requires an

Irradiance of 500 μ mol·m−2·s−1 for Greatest Edible Biomass Production. *HortScience*, *44*(1), 64–67.

Brodersen, C. R., & Vogelmann, T. C. (2010). Do changes in light direction affect absorption profiles in leaves? *Functional Plant Biology*, *37*(5), 403–412.

Chen, X. L., Xue, X-z, Guo, W-z, Wang, Li-c, & Qiao, X-j. (2016). Growth and nutritional properties of lettuce affected by mixed irradiation of white and supplemental light provided by light-emitting diode. *Scientia Horticulturae*, *200*, 111–118.

Folta, K. M. (2004). Green Light Stimulates Early Stem Elongation, Antagonizing Light-Mediated Growth Inhibition 1. *Plant Physiology*, *135*, 1407–1416.

Folta, K. M. (2005). Green Light Effects on Plant Growth and Development. In n: Wada M., Shimazaki K., Iino M. (Ed.), *Light Sensing in Plants* (pp. 239–242). Tokyo: Springer.

Folta, K. M., & Maruhnich, S. A. (2007). Green light: a signal to slow down or stop. *Journal of Experimental Botany*, *58*(12), 3099–3111.

Hovi-Pekkanen, T., & Tahvonen, R. (2008). Effects of interlighting on yield and external fruit quality in year-round cultivated cucumber. *Scientia Horticulturae*, *116*(2), 152–161.

Inada, K. (1976). Action spectra for photosynthesis in higher plants. *Plant and Cell Physiology*, *17*(2), 355–365.

Johkan, M., Shoji, K., Goto, F., Hahida, S., & Yoshihara, T. (2012). Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in Lactuca

sativa. *Environmental and Experimental Botany*, *75*, 128–133.

Kaiser, E., Weerheim, K., Schipper, R., & Dieleman, J. A. (2019). Partial replacement of red and blue by green light increases biomass and yield in tomato. *Scientia Horticulturae*, *249*, 271–279.

Kami, C., Lorrain, S., Hornitschek, P., & Fankhauser, C. (2010). Light-Regulated Plant Growth and Development. *Current Topics in Developmental Biology*, *91*, 29–66.

Kang, Y. R., Park, J., Jung, S. K., & Chang, Y. H. (2017). Synthesis, characterization, and functional properties of chlorophylls, pheophytins, and Zn-pheophytins. *Food Chemistry*, *245*, 943–950.

Kim, H., Goins, G. D., Wheeler, R. M., & Sager, J. C. (2004). Green-light Supplementation for Enhanced Lettuce Growth under Red- and Blue-light-emitting Diodes. *HortScience*, *39*(7).

Macedo, A. F., Leal-Costa, M. V., Tavares, E. S., Lage, C. L. S., & Esquibel, M. A. (2011). The effect of light quality on leaf production and development of in vitro-cultured plants of Alternanthera brasiliana Kuntze. *Environmental and Experimental Botany*, *70*(1), 43–50.

Mandoli, D. F., & Briggs, W. R. (1981). Phytochrome control of two low-irradiance responses in etiolated oat seedlings. *Plant Physiology*, *67*(4), 733–739.

McCree, K. J. (1971). The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agricultural Meteorology*, *9*, 191–216.

Meng, Q., Kelly, N., & Runkle, E. S. (2019). Substituting green or far-red radiation for

blue radiation induces shade avoidance and promotes growth in lettuce and kale. *Environmental and Experimental Botany*, *162*, 383–391.

Mickens, M. A., Skoog, E. J., Reese, L. E., Barnwell, P. L., Spencer, L. E., Massa, G. D., & Wheeler, R. M. (2018). A strategic approach for investigating light recipes for 'Outredgeous' red romaine lettuce using white and monochromatic LEDs. *Life Sciences in Space Research*, *19*, 53–62.

Mickens, M. A., Torralba, M., Robinson, S. A., Spencer, L. E., Romeyn, M. W., Massa, G. D., & Wheeler, R. M. (2019). Growth of red pak choi under red and blue, supplemented white, and artificial sunlight provided by LEDs. *Scientia Horticulturae*, *245*, 200–209.

Nishio, J. N. (2000). Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. *Plant, Cell and Environment*, *23*(6), 539–548.

Olle, M., & Viršile, A. (2013). The effects of light-emitting diode lighting on greenhouse plant growth and quality. *Agricultural and Food Science*, *22*(2), 223–234.

Possart, A., Fleck, C., & Hiltbrunner, A. (2014). Shedding (far-red) light on phytochrome mechanisms and responses in land plants. *Plant Science*, *217–218*, 36–46.

Sager, J. C., Smith, W. O., Edwards, J. L., & Cyr, K. L. (1988). Photosynthetic Efficiency and Phytochrome Photoequilibria Determination Using Spectral Data. *Transactions of the ASABE*, *31*(6), 1882–1889.

Smith, H. (1982). Light Quality, Photoperception, and Plant Strategy. *Annual Review of Plant Physiology*, *33*(1), 481–518.

Smith, H. L., McAusland, L., & Murchie, E. H. (2017). Don't ignore the green light: exploring diverse roles in plant processes. *Journal of Experimental Botany*, *68*(9), 2099–2110.

Smith, H., & Whitelam, G. C. (1997). The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. *Plant, Cell and Environment*, *20*(6), 840–844.

Snowden, M. C., Cope, K. R., & Bugbee, B. (2016). Sensitivity of Seven Diverse Species to Blue and Green Light: Interactions with Photon Flux. *PloS One*, *11*(10).

Song, Y. H., Ito, S., & Imaizumi, T. (2013). Flowering time regulation: photoperiod- and temperature-sensing in leaves. *Trends in Plant Science*, *18*(10), 575–583.

Sun, J., Nishio, J. N., & Vogelmann, T. C. (1998). Green Light Drives CO 2 Fixation Deep within Leaves. *Plant Cell Physiol*, *39*(10), 1020–1026.

Talbott, L. D., Nikolova, G., Ortiz, A., Shmayevich, I., & Zeiger, E. (2002). Green light reversal of blue-light-stimulated stomatal opening is found in a diversity of plant species. *American Journal of Botany*, *89*(2), 366–368.

Terashima, I., Fujita, T., Inoue, T., Chow, W. S., & Oguchi, R. (2009). Green Light Drives Leaf Photosynthesis More Efficiently than Red Light in Strong White Light: Revisiting the Enigmatic Question of Why Leaves are Green. *Plant and Cell Physiology*, *50*(4), 684–697.

Wellburn, A. R. (1994). The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different

Resolution. *Journal of Plant Physiology*, *144*(3), 307–313.

Zhang, G., Shen, S., Takagaki, M., Kozai, T., & Yamori, W. (2015). Supplemental Upward Lighting from Underneath to Obtain Higher Marketable Lettuce (Lactuca sativa) Leaf Fresh Weight by Retarding Senescence of Outer Leaves. *Frontiers in Plant Science*, *6*, 1110.

Zhang, T., Maruhnich, S. A., & Folta, K. M. (2011). Green light induces shade avoidance symptoms. *Plant Physiology*, *157*(3), 1528–1536.