



Horticultural Fellowship Awards

Interim Report Form

Project title: Securing skills and expertise in crop light responses for UK protected horticulture, with specific reference to exploitation of LED technology (EMT/AHDB Horticulture/HTA Fellowship)

Project number: CP 085

Project leader: Dr G M McPherson, STC

Report: Annual report, November 2014

Previous report: N/A

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Location of project: Stockbridge Technology Centre

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Neal Wright, Micropropagation

Services

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Colin Frampton, Consultant

Steve Carter, Protected and Indoor
Ornamentals

Geoffrey Smith, Mapleton Growers
(Protected Edibles - Lettuce)

Date project commenced:

01 October 2012

Date project completed

30 September 2017

(or expected completion date):

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr Phillip Davis

Project Manager

Stockbridge Technology Centre

Signature Date

Report authorised by:

Dr Martin McPherson

Science Director

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Signature Date

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Progress Against Objectives

Objectives

Training

Objective T1. To provide the Fellow with the knowledge, understanding and practical skills to undertake applied plant science in the area of plant light responses, lighting and cladding materials.

Objective T2. To establish the Fellow with a network of contacts within the major commercial producers of horticultural LEDs (and cladding plastics).

Objective T3. To establish the Fellow with a network of contacts in the science base in fundamental and applied plant photobiology in Europe and beyond.

Objective T4 To provide the Fellow with a solid appreciation of the “business basis” for horticultural R&D, including aspects such as staffing, costings and the range of possible funding routes.

Objective T5. To establish the Fellow with a network of contacts with experts from the industry, including applied horticultural consultants currently active in supporting UK protected cropping, and through their respective technical groups, representatives of the major protected cropping sectors in the UK.

Research

Objective R1. For the fellow to undertake an initial, objective review of current developments and progress in lighting technology with support from the leading manufacturers and including a brief fact-finding tour overseas.

Objective R2. To objectively assess the properties of a selection of LEDs currently available or proposed for use in UK horticulture in terms of total irradiance (intensity), spectrum, efficiency and response to dimming.

Objective R3. Based on R1 and R2 to identify gaps in current scientific knowledge with respect to crop responses (using existing *Arabidopsis* light response knowledge) to LEDs relevant to UK production and to undertake pilot-scale experiments into the responses of selected UK protected crops (particularly leafy salads, ornamentals & herbs) to LEDs found

to have useful properties in R2. Information obtained under R3 will identify the potential of appropriate lighting systems for specific UK crops. The knowledge gained will be used to design further R&D studies, subject to additional external funding, for future commercial implementation in the UK (See Objective R5).

Objective R4. In addition to the Fellowship reports, to produce (i) a technical review of the “state-of-art” of LED lighting in Horticulture, (ii) an article in AHDB Grower summarising the results of the Fellowship and the current status of LED lighting in horticulture and (iii) to participate in a programme of visits, workshops and conferences for growers, including those at the new STC facility.

Objective R5. As noted above (see Research aims), this fellowship is intended as being a major element in securing long-term R&D in to LEDs (and other light-based approaches to production) in UK horticulture, but does not in itself deliver a major “stand-alone” research programme. AHDB Horticulture is currently undertaking a major assessment of future priorities for UK-based LED research, and while the outcome of this review remains uncertain, we anticipate that, based on progress during the fellowship and other projects, the Fellow will be taking the lead in preparing applications for funding to extend R&D in LED lighting in the UK, with the Fellow as the lead investigator.

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
Objective T1.	December 2012	December 2012	-
Objective T2.	December 2012	This is an on-going exercise as new companies move into the area.	September 2017
Objective T3.	December 2013	Many contacts have been made but this is an on-going exercise that will run throughout the	September 2017

		project.	
Objective T4.	December 2013	This process will continue throughout the fellowship.	September 2017
Objective T5.	December 2013	This process will continue throughout the fellowship.	September 2017
Objective R1.	December 2012	February 2013	-
Objective R2.	January 2013	March 2013	-
Objective R3.	December 2013	-	- December 2014
Objective R4.	December 2016	-	-
Objective R5.	September 2017	-	-

Summary of Progress

The second year of the Fellowship has progressed well and a substantial amount of knowledge on crop production under LED lighting systems has been gained.

Training objectives

Objective T1.

Completed year one.

Objective T2.

During the second year of the fellowship, I made new contacts with staff at Phytolux and have remained in contact with other LED manufacturers met during the first year. I have also been able to examine LED panels from an additional company, Solidlite, and will be testing plant growth under a range of manufacturers' lights as part of the AHDB Horticulture CP125 project. Via the development of a new LED glasshouse facility for high-wire crops at STC, I have also had the opportunity to examine the new Philips LED HPS replacement lamps as well as their inter-lighting systems. In future years I will remain alert to advances in the LED market and make contacts with new companies where appropriate. During the second year of the fellowship, I also initiated a project to examine plastic covering materials. I obtained samples with a view to becoming familiar with the range of products that are available and to examine the spectral properties of these materials. Substantial assistance

regarding plastic covering materials was received from the company 'NP Structures', who also provided the Solidlite LED panels I will be testing.

Objective T3.

During the second year of the Fellowship I continued to meet and develop links with additional scientists worldwide. I have also made progress with the collaborations initiated during year one of the fellowship program. I am a co-supervisor for Mr Richard Boyle, (one of Dr. Ian Dodds' PhD students at Lancaster University. As part of this, I grew plants for Mr. Boyle and hosted his week-long visit to STC to allow him to perform a range of physiological measurements. This work will be repeated early in 2015. Via contacts in the US, Prof Roberto Lopez and Prof Eric Runkle, I have been asked to be a member of the Scientific Committee for the 8th ISHS International Symposium on Light in Horticultural Systems. Finally, during a visit to Prof Carl-Otto Ottosen's laboratory in Denmark, I performed experiments examining the influence of light colour of the amount of light stress experienced by plants. These experiments are described in detail latter in this report.

Objective T4.

During the second year of the fellowship, I began to run the AHDB Horticulture CP125 research program. This has provided an opportunity to learn how to manage large research programs while remaining within budget. I have also improved my personnel management skills with regard to time management and also by training staff to perform tasks efficiently and to the correct standards. I have also remained up to date with potential new funding opportunities and am working with several universities with the aim of attracting additional funding for further collaborative research projects.

Objective T5.

Through the grower visits organised via the fellowship program I have made new contacts and learned more about the diversity of nurseries within the UK, especially in the ornamental sector. During a visit to the South Coast, organised by Mr Colin Frampton, I visited VHB Herbs, Madestein Ltd, Roundstones, Hill Bros Ltd, Tangmere Airfield Nurseries Ltd., and Hall Hunter. I also continued to make contacts through day-to-day activities at STC, such as via commercial activities and through hosting visits to the LED4CROPS research facility and STC's new LED glasshouse facility for high-wire crop production. During the third year of the fellowship program I will focus on visiting hardy nursery stock sites. An analysis of the contacts made in the different sectors is included in Appendix 1.

Research Objectives

Objectives R1 & R2.

I have maintained an up-to-date perspective of the range of LED lights available for horticulture and, where possible, have made spectral assessments of those lights. I have also been encouraged by various growers to develop methods to assess/predict plant responses to different LED lights. With this in mind, I have begun to develop a range of methods to analyse light quality and to make physiologically based predictions of how plants respond to different light qualities. These methods will be used to assist the AHDB Horticulture CP125 project to describe/understand plant responses to different light spectra. These tools will continue to be developed and used to help growers when appropriate.

Objective R3.

Research projects running in the LED4CROPS facility allowed me to further develop my knowledge of crop production in the different sectors of the industry. This prompted the identification of areas where more research is required and helped focus the goals of the AHDB Horticulture CP125 research program. This research project has already provided interesting data that could lead to sector-specific best practice protocols. While the experiments primarily focused on LED lighting, some of the earlier outcomes may be achievable with spectral filters. My ongoing aim will be to keep growers up to date with progress and, where appropriate, develop spin-off projects to rapidly develop improved protocols for growers.

Objective R4.

The technical review of lighting in horticulture has been delayed due to the fast moving nature of technological developments in this field and the additional work load caused by the AHDB Horticulture CP125 project. Two AHDB Grower articles will be produced one for the end of 2014 and one for early 2015. In addition, I attended a wide range of grower and scientific conferences and workshops, and presented at several of these (see training and knowledge transfer sections for full details).

Objective R5.

During the first year of the fellowship I secured funding for a “stand-alone” AHDB Horticulture-funded project to examine plant light responses (AHDB Horticulture project CP125). This research project has been underway for six months and has provided numerous training opportunities as well as additional information regarding plant light responses. To date, the project has examined light responses in herbs (basil, mint, sage), cucumber and a trial examining the potential of LED lights to improve the rooting of hardy

nursery stock cuttings (Photinia, Elaeagnus and Rhododendron) is underway. The next trial will examine the light responses of a range of bedding plants. In addition to the plant work, the project will examine insect responses under LED lighting. This work package will be performed by the entomology team at STC. This project is allowing me to further develop the skills required to manage larger and more diverse project teams and to ensure a co-ordinated approach to ensure timely project delivery.

Milestones not being reached

The 'State of the Art of LED Lighting' technical review delivery deadline has been delayed until March 2015.

Do remaining milestones look realistic?

Yes

Training undertaken

Conferences attended

UV4Growth Odense, Denmark, March 2014 - Speaker.

Vertical Growing and Urban Agriculture Conference, Nottingham University, September 2014 - Speaker.

AHDB Horticulture Studentship Conference, York, September 2014.

Tomato Growers Annual Meeting, Coventry, September 2014.

Grower visits

Plant Raisers, Tomato and bedding plant propagator, November 2013.

Watts Farm, Herb grower, December 2013

Hall Hunter, Soft Fruit, May 2014.

Tangmere Airfield Nurseries, Pepper Grower, May 2014.

Roundstone Nurseries, Bedding Plants, May 2014.

Madestein Ltd, Herb grower, May 2014.

Hill Bros, Bedding Plants, May 2014.

VHB Herbs, Herb grower, May 2014.

Other

Grant writing course

Training session with Derek Hargreaves covering irrigation and nutrient solutions.

Expertise gained by trainees

I have continued to develop my knowledge of the available LED lighting systems as well as spectral filters.

Other achievements in the last year not originally in the objectives

I have increased my breadth of industry knowledge through additional grower visits. Interaction with growers has helped me develop an understanding of how the results from LED projects can impact a wider range of growers, including those not using LEDs, via spectral manipulation with filters.

My project and personal supervision skills are developing to match the needs of the range and number of projects that I continue to manage in the STC LED4CROPS facility.

Changes to Project

Are the current objectives still appropriate for the Fellowship?

Yes

GROWER SUMMARY 1

Light spectrum and plant stress.

Headline

Blue light stresses plants more than red or green light. Altering the light spectrum could improve plant growth rates by reducing stress or improving stress tolerance; this could reduce the time required to harden off plants prior to transplanting.

Background

To prevent leaf scorching, plants generally need to be hardened off when they are moved from a protected environment to a field setting. Scorch can be caused by several factors, one of which is light stress (photoinhibition). Plants acclimate to the amount of light they receive; this allows them to photosynthesize at maximum efficiency in a given environment. Part of the acclimation process involves increasing the rate of repair of photoinhibition to match the rate of photoinhibition occurring in the plant. If exposed to a sudden increase in light level, plants may be unable to use all the light for photosynthesis. The excess light energy may then cause photoinhibition and lead to scorching. A sudden decrease in temperature can also cause the same effect because photosynthesis occurs more slowly in lower temperatures.

Different colours of light can result in different amounts of stress. UV light in particular can cause extensive damage when plants are moved outdoors as many covering materials (glass and polythene covers) filter UV light. Plants produce protective pigments on exposure to UV light, but leaves will be damaged if exposure is high before sufficient concentrations of these pigments can be produced.

It is possible to select the colour of light to meet the needs of plants using LEDs. It should, therefore, be possible to select a light spectrum that can reduce light stress and maximise photosynthetic efficiency. Alternatively, a light spectrum could be selected that stresses plants in a controlled manner to help the hardening off process or encourage the synthesis of secondary metabolites such as anthocyanin (the pigment that turns leaves red). A necessary first step in determining the feasibility of these approaches is the determination of the amount of light stress caused by different colours of commercially available LED light. This experiment was performed during a one week visit to Prof Carl-Otto Ottosen's laboratory at Aarhus University, Denmark.

Summary

Experiments were performed to assess how rapidly blue, green, and red light from LED lighting systems cause photoinhibition. To do this, plants were treated with lincomycin, a chemical that prevents plants from repairing the damage caused by light stress. Leaves were then exposed to LED light of different colours and the rate of damage tracked through time. The amount of photoinhibition was measured using a chlorophyll fluorimeter. This instrument measures a parameter referred to as F_v/F_m . When this value is close to 0.84, no photoinhibition has occurred. Values decrease towards zero as photoinhibition occurs. Photoinhibition was found to be greatest when plants were exposed to blue LED light and least when exposed to green LED light. The experiment also assessed the gradient of photoinhibition that occurs between the upper and lower surface of leaves. Light intensity decreases as it passes through a leaf, and more damage should therefore occur near the upper surface. As expected, more damage occurred near the upper surface of the leaves where the light intensity was greatest. The difference between the damage occurring between the upper and lower leaf surfaces was strongly influenced by light colour. The difference was greatest for blue light and least for green light, as would be expected given the greater leaf penetration of green light.

Financial Benefits

It is not possible, as this stage, to assess the potential financial implications of this research. This work would be expected to help the ongoing development of a lighting spectrum designed to maximise plant growth rates and/or to assist the hardening off process.

Action Points

It is too early in this program of research to make recommendations for best practice in use of light spectrum manipulation for improving stress tolerance.

SCIENCE SECTION

Light spectrum and plant stress

Introduction

Photosynthesis within plants occurs within chloroplasts, which are small structures found within plant cells. Chloroplasts contain all the pigments and proteins required to perform photosynthesis, the process by which plants use light to harvest CO₂, generate carbohydrates, and produce oxygen. Photosynthesis consists of two major phases, often referred to as the 'light' and 'dark' reactions. Each of these phases consists of a series of complex reactions. During the 'light' reactions, photons (light) are absorbed by chlorophyll and their energy is passed to the reaction centres (multi-protein complexes). Plants have two types of reaction centre, photosystem one (PSI) and photosystem two (PSII). PSII uses light energy to split water molecules (oxygen is produced as a by-product). Electrons are generated during this process, and these are passed to PSI. PSI then uses light energy to pass the electrons to NADP⁺ molecules, which become converted to NADPH in the process. The light reactions also generate ATP (the energy currency of all living cells). The 'light' reactions can only occur when leaves are illuminated as each step of the process is activated by light. The 'dark' reactions, however, can operate whenever ATP and NADPH are available and can therefore occur in light or in darkness. The NADPH and ATP produced by the 'light' reactions convert CO₂ to carbohydrates via the Calvin-Benson cycle.

The light reactions of photosynthesis produce a series of highly energetic compounds that can cause damage to organic compounds and tissues. The majority of the absorbed light energy is used in photosynthesis, but a small proportion remains unused and has the potential to cause damage. PSII is particularly sensitive to damage during illumination. Damage within PSII is generally confined to a single protein, which is called D1. When the D1 protein is damaged, PSII is disassembled and reconstructed with a newly-synthesised D1 protein. When exposed to light, plants must continuously repair the damage to PSII in order to maintain efficient photosynthesis and prevent the production of reactive oxygen species. Plants are able to adjust the rate of PSII repair to match the local environmental conditions. However, when exposed to stress conditions, e.g. high light, low temperature, or drought, the rate of damage can exceed the rate of repair and photosynthesis and growth rates will be reduced. In extreme cases, photoinhibition can lead to leaf damage or even crop death.

Photoinhibition occurs in the presence of any light but different colours of light result in different rates of damage. Red light is less damaging than blue light and blue light is less damaging than UV light. Using LEDs, it may be possible to select colours that minimise photoinhibition while maximising photosynthetic and growth rates as well as optimising plant qualities like flavour and pigmentation.

The amount of PSII damage can be assessed by using a chlorophyll fluorimeter to measure the dark-acclimated F_v/F_m parameter. Under non-stressed conditions, a dark-acclimated leaf should have a F_v/F_m value close to 0.84. This value decreases as PSII damage occurs, reaching 0 when all the PSII complexes are damaged. The rate of damage cannot be assessed while leaves are continually repairing PSII. However, the rate of PSII damage can be assessed if the repair mechanism is deactivated by the application of an inhibitor (e.g., lincomycin or chloramphenicol)

In this experiment, the rate of photoinhibition was determined in lincomycin-treated spinach leaves illuminated with different colours of LED light. These experiments were performed during a one-week visit to Prof. Carl Otto-Ottosen's laboratory in Aarhus University, Denmark.

Materials and methods

Plant material

Spinach plants were grown in the Aarhus University glasshouse during November and December 2013. The glasshouse was heated to 18°C and supplemental light was provided using high-pressure sodium (HPS) lights for a period of 16 hours. Plants were grown in 10cm pots containing a peat based compost and were irrigated once per day using an automated ebb and flood irrigation system.

Light treatments

Three different colours of LED light were used for the inhibition experiments: red (660 nm), green (530 nm) and blue (450 nm). Blue inhibitory light was provided by Fionia FL300 lamps while other colours were provided by Heliospectra L4A series 10 lamps. Light spectra were measured using an Ocean optics Jaz spectroradiometer, see figure 1. The light intensities were ~650, ~1200, and ~500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the blue, red, and green light sources, respectively. The total light dose (D) received by each leaf section was defined as the light intensity directly above the leaf multiplied by the duration of exposure in seconds. Light dose units were mol m^{-2} and were equivalent to a daily light integral. To demonstrate that lincomycin treatment did not itself cause PSII photoinhibition, control leaves were

treated with lincomycin but were not exposed to inhibitory light. These leaves functioned normally.

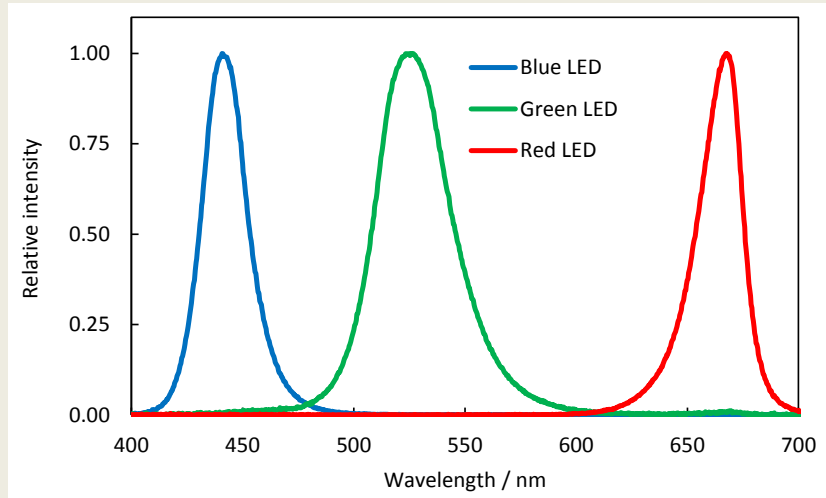


Figure 1. Spectra of the three LED light sources used in inhibition experiments.

Photoinhibition protocol

Leaves were detached from the plants and cut into sections, of at least 6 cm², that would lie flat when placed on a surface. Leaf sections were vacuum-infiltrated with 5.4 mM lincomycin (Sigma-Aldrich, New Road, Gillingham, Dorset, SP8 4XT). After vacuum infiltration, leaf sections were placed in dim light until all the intercellular liquid had been absorbed. A SPAD 502 plus chlorophyll meter was used to assess the chlorophyll content of each leaf section which were subsequently placed in the upturned lid of a petri dish. The base of the petri-dish was then placed on top of the leaf to hold the section flat. A small amount of lincomycin solution was added to keep the leaf hydrated during the experiments. Petri dishes were then placed under the inhibitory light, see figure 2. The light intensity directly above each leaf section was measured using a LiCor PAR meter. After exposure for a fixed amount of time the leaf sections were dark acclimated for 30 minutes, after which the F_v/F_m values of the upper and lower leaf surfaces were measured using a Walz minipam (Heinz Walz GmbH, Eichenring 6, 91090 Effeltrich, Germany). Following F_v/F_m measurement the leaves were returned to the same location under the light source. The change in F_v/F_m of each leaf section was tracked through time with experiments running for up to 36 hours.

The rate of PSII inhibition was modelled with the equations:

$$F_v/F_m = 1e^{-k_I D} \quad \text{for } D < D_T \quad (1)$$

$$F_v/F_m = [1e^{-k_I D}]e^{-k_c(D-D_T)} \quad \text{for } D > D_T \quad (2)$$

where D is the light dose ($\text{mol}\cdot\text{m}^{-2}$), D_T is the threshold light dose at which the rate of inhibition changes, k_l is the decay rate of F_v/F_m for light doses lower than D_T and k_c is the decay rate of F_v/F_m for light doses greater than D_T .



Figure 2. Experimental setup showing leaves in petri dishes located directly under one of the Heliospectra lamps.

Results

The mean F_v/F_m values measured from the upper and lower leaf surfaces of control leaves (those left in darkness following lincomycin treatment) were 0.82 and 0.83, respectively. No decrease in F_v/F_m was observed in control leaves even 36 hours after the lincomycin treatment was applied.

For the leaves exposed to light, F_v/F_m values decreased rapidly as photosystem II (PSII) became progressively damaged. The F_v/F_m value decay occurred in a non-linear response that most closely resembled an exponential function. The rate of decrease was proportional to the cumulative light exposure or light dose (Figure 3) as well as the colour of the light. The decay was observed to occur over two distinct phases that were separated by a threshold light dose, where the rate of decay increased at light doses greater than the threshold value. The threshold light dose was different for each colour of light and occurred at the lowest light dose for blue light and at the highest light dose for red light, see table 1.

The rate of F_v/F_m decay measured from the upper leaf surface was greater than that measured for the lower leaf surface. Cells near the lower leaf surface were shaded by the cells near the upper surface and were, therefore, exposed to a lower light intensity/dose.

The difference between the amount of photoinhibition that occurred at the leaf upper and lower surfaces was also different for the three colours of light, see Figure 2A–2C, especially when examined for light doses up to 10 mol.m⁻². These differences were assessed by

Table 1. Model parameters for the photoinhibition of PSII in lincomycin-treated leaves exposed to blue, green, or red LED light. Models were fitted to the raw data using the least squares method. Values for upper and lower leaf surface F_v/F_m measurements are shown.

Colour	Upper surface			Lower surface		
	K_1	K_C	$D_T / \text{mol m}^{-2}$	K_1	K_C	$D_T / \text{mol m}^{-2}$
Blue	0.043	0.077	8.	0.017	0.056	9.1
Green	0.017	0.046	11.0	0.011	0.030	8.9
Red	0.034	0.065	33.8	0.019	0.036	32.2

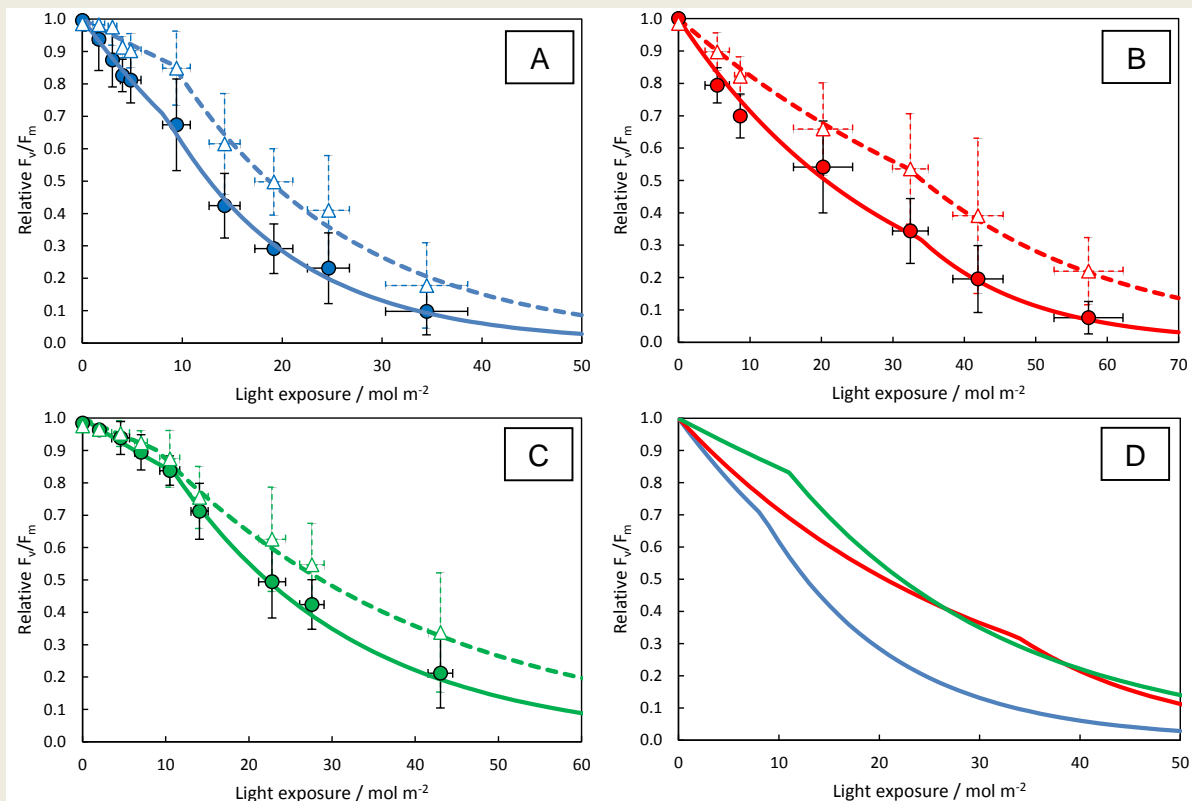


Figure 3. The rate of photoinhibition occurring on lincomycin-treated spinach leaves following exposure to blue (A), red (B), or green light (C). Photoinhibition was determined for the upper (solid symbols and lines) and lower (open symbols and dashed line) leaf surfaces. D) Comparison of modelled inhibition curves for the upper leaf surfaces of leaves

exposed to the three colours of light. Each data point represents the mean of 5–10 measurements and error bars indicate the standard deviation.

calculating the differences between the modelled rates of F_v/F_m decay of the upper and lower surfaces, see Figure 4. The modelled difference in inhibition between leaf surfaces for blue light increased rapidly with increasing light dose, indicating a large difference between the amounts of photoinhibition occurring at the two surfaces. The difference between the models peaked at about 10 $\text{mol}\cdot\text{m}^{-2}$ before declining. The modelled differences calculated for the red and green spectra peaked at approximately 30 $\text{mol}\cdot\text{m}^{-2}$ and changes with respect to increasing light intensity were lower than for blue light. The modelled differences were lowest with green light, particularly during the first phase of inhibition. This indicated that more similar amounts of photoinhibition occurred at the upper and lower surfaces of the leaf when treated with green light compared to red and blue light.

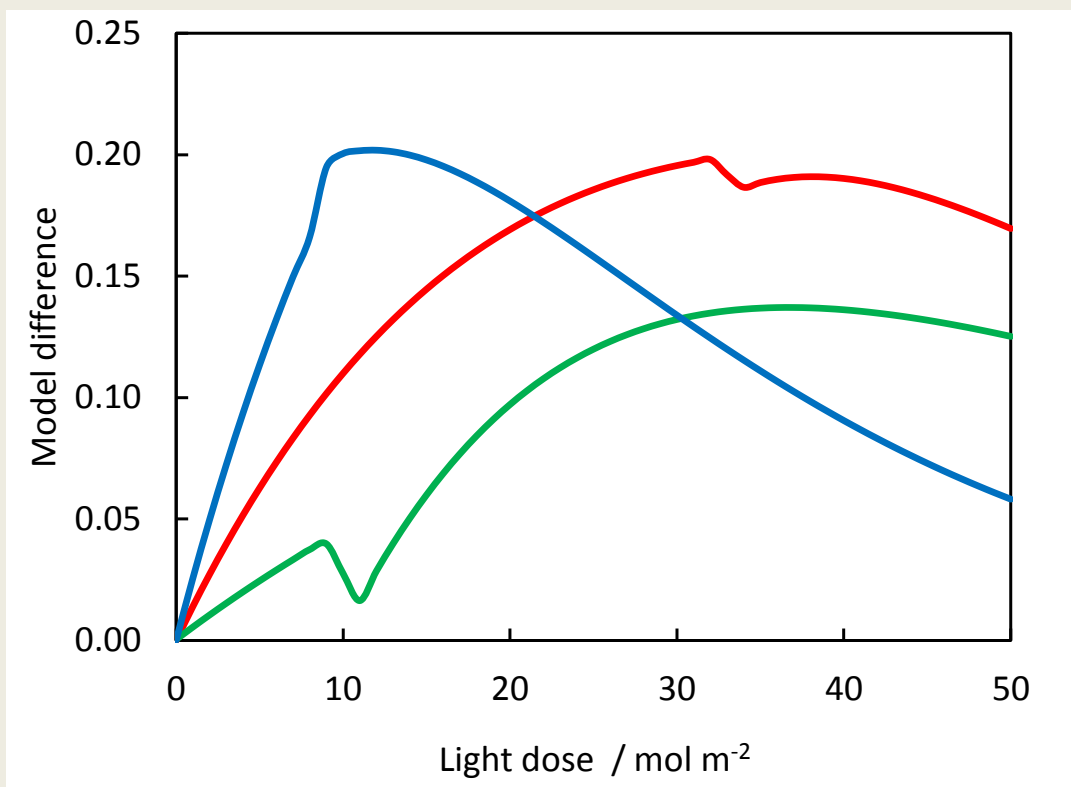


Figure 4. Differences between inhibition models for upper and lower leaf surfaces. Blue line, values calculated for inhibition with blue light; green line, values calculated for inhibition with green light; red line, values calculated for inhibition with red light.

Discussion

In this experiment, the rate of PSII photoinhibition was greatest under blue light. These results are consistent with previous studies examining the rate of photoinhibition under different colours of light, which all found blue light to be more damaging than green or red light. In experiments examining the spectrum of inhibition, the rate of PSII inhibition was found to increase as the colour of light changed from blue to UV (Sarvikas *et al* 2006, Takahashi *et al* 2010). UV light with wavelengths of 254 and 365nm caused greater than 50 times more damage than green light (Sarvikas *et al* 2006). While the photoinhibition caused by UV light is consistently greater than visible light there is more variability in the estimates of inhibition received by leaves exposed to longer wavelengths of light (green, yellow, and red light). Sarvikas *et al* (2006) reported little variation in the rate of inhibition occurring at wavelengths of 450–700nm, while Takahashi *et al* (2010) observed that the rate of inhibition peaked at 600nm (yellow light). Oguchi *et al* (2011) found the rate of inhibition to be lower in green than in red light. Our results also suggest that red light causes more inhibition than green light. While the relative amounts of inhibition caused by red, yellow, and green light may cause differences in photosynthetic efficiency, the main inhibition is caused by blue and UV light. Blue and UV light are both known to induce the synthesis of pigments that can protect plants from blue and UV photoinhibition. This means that any light recipe designed to increase plant stress tolerance by controlled increase of photoinhibition rates and, therefore, up-regulate repair mechanisms, will have the added benefit of increasing production of the protective chemicals.

The dual-phase model used to determine the rates of inhibition in this experiment was proposed by Davis & Hangarter (2012). This suggested that, at the threshold light-dose (D_T), the rate of inhibition increases because cyclic electron flow around photosystem I (PSI) fails. This assumes that cyclic electron flow around PSI has a role in protection of PSII (Takahashi *et al.* 2009). In the current experiment, the point at which the inhibition rate increased occurred at different light-doses for the three colours of light. This suggests that the different colours of light might induce different amounts of cyclic electron flow. However, it cannot be ruled out that the varied intensities of the inhibitory lights might be responsible for the observed differences.

In addition to demonstrating that blue light caused more photoinhibition/stress to plants than red or green light, these experiments also showed the effect of wavelength on leaf light penetration. When inhibition occurring on the upper and lower surfaces of leaves was compared (Figure 4), the difference between the values varied with light wavelength (green<red<blue). This concurred with previous studies that examined the gradients of light

within leaves (Vogelman *et al* 1996) and gradients of photoinhibition within leaves (Oguchi *et al* 2011). While important for the amount of photoinhibition, the differences in light penetration between the different colours will also impact the ability of leaves to photosynthesize. For example, green light can penetrate further into leaves than blue light and can, therefore, drive photosynthesis more effectively deep within the leaf (Terashima *et al*, 2009).

Blue light causes chloroplasts to move within cells. Under low light, chloroplasts accumulate at the upper and lower surfaces of cells, thereby maximising light absorptance. Under bright light, chloroplasts move to the sides of cells, thereby maximising light penetration. These movements were previously shown to alter the gradient of photoinhibition within leaves (Davis and Hangarter, 2012). In the present study, chloroplast movement was allowed to progress normally, and it is therefore likely that chloroplasts were arranged differently in the blue light treatment compared to the red and green light treatments. Despite this likely arrangement, which would increase light penetrance though the leaf in blue light, photoinhibition remained higher for blue light treatments than other treatments.

Conclusions

Blue light causes more photoinhibition than red or green light. This information will aid development of light recipes that minimise plant stress and, therefore, maximise growth rates. Conversely, these results may also be of value in developing light recipes designed to increase stress tolerance and potentially reduce or remove the need to harden off plants prior to transplanting.

GROWER SUMMARY 2

Influence of far-red light on the morphology and development of a range of herb species.

Headline

Far-red light induces plant stretching, leaf curling and flowering. The magnitude of these responses differs between species.

Background

Poor light levels can prompt both stretching and flowering in plants. This shade-avoidance response is induced by low intensities of blue and red light and relatively high intensities of far-red light. In general, blue and red light causes plants to remain compact, while far-red light causes plants to stretch and flower. While these general rules can be applied to most plant species, the relative magnitude of the responses varies considerably. With LED lighting it is possible to carefully manipulate the quality of light that plants are exposed to and, in doing so, we can examine the diversity and magnitude of plant responses to light. Herb crops come from an extensive range of habitats and plant lineages and would be expected to show a wide range of responses to light. A thorough understanding of light responses within this group would help to grow better plants and classify plants with regard to their lighting requirements.

This small scale trial was aimed at examining the influence of far-red LED on herb plant morphology. The focus of the work was to make qualitative assessments of the diversity of plant light responses to help focus more refined future research projects and ensure the correct parameters are measured. The results will also function as a range-finding exercise for light recipe development.

Summary

Far-red light caused an increase in internode length and plant height in most species. The exception to this was Sage, in which internode lengths were equal for plants grown with and without far-red light. The greatest response to far-red light was observed in Dill. Dill plants grown with far-red light were three times taller than those grown without far-red light. The morphology of the far-red grown plants was extremely stretched and flowering occurred. Dill plants grown without far-red remained extremely compact. Bronze fennel plants were next most sensitive to far-red light; stems were 4.7 times longer but the plants did not flower when grown with far-red light than without far red. The bronze fennel plants did not flower during this experiment. Mint and Thyme showed moderate responses to far-red light, with

increases in plant height or internode length of 1.2–1.8 times those of plants grown without far-red light.

Leaf morphology responses to far-red varied between species. Thyme demonstrated leaf curling and the mint leaves became curled and blistered in appearance. Sage leaves became narrower but demonstrated no curling. The leaves of the Dill and Bronze fennel plants became more feathery in appearance.

Financial Benefits

It is too early in this program to assess the potential financial aspects of the results.

Action Points

Further work is required to refine light recipes to enhance the different aspects of herb crops. The results from this experiment will feed into and guide the experiments planned in the AHDB Horticulture CP125 research project. These experiments will further our understanding of plant responses to different red:far-red ratios provided by LEDs.

Further work is required to understand how the red:far-red ratio of light provided by LEDs compares to the red:far-red ratio of other lighting systems with regards to plant morphology and development.

SCIENCE SECTION

Influence of far-red light on the morphology and development of a range of herb species. Introduction

In low-light conditions many plants grow taller to maximise their chance of reaching the higher-light environments that will improve growth rates and survival chances. When plants are shaded by other plants, they receive a lower level of light. However, in addition, higher leaves filter the light and this also changes the light quality. Sunlight has a red:far-red ratio of close to 1. As light passes through a canopy, proportionally more of the red and blue light is absorbed and proportionally more of the green and far-red penetrates downwards. This causes a reduction in the red:far-red ratio of light lower in the canopy.

Plants sense the red:far-red ratio of light using phytochromes (a class of photoreceptors). Plants possess several types of phytochrome. The functions of the different members of the family are slowly being elucidated but the majority of the research to date has focused on two types of phytochrome, phyA and phyB, which seem to be responsible for the majority of the morphological responses to red:far-red light. PhyA and PhyB have different responses to light. PhyA is activated by both red and far-red light whereas PhyB is activated by red light but deactivated by far-red light. PhyA and PhyB therefore provide plants with the ability to sense different wavelengths of light. PhyA is therefore likely to be active under all light treatments used in this study, and any response will thus be caused by the addition of far-red light and the activity of PhyB.

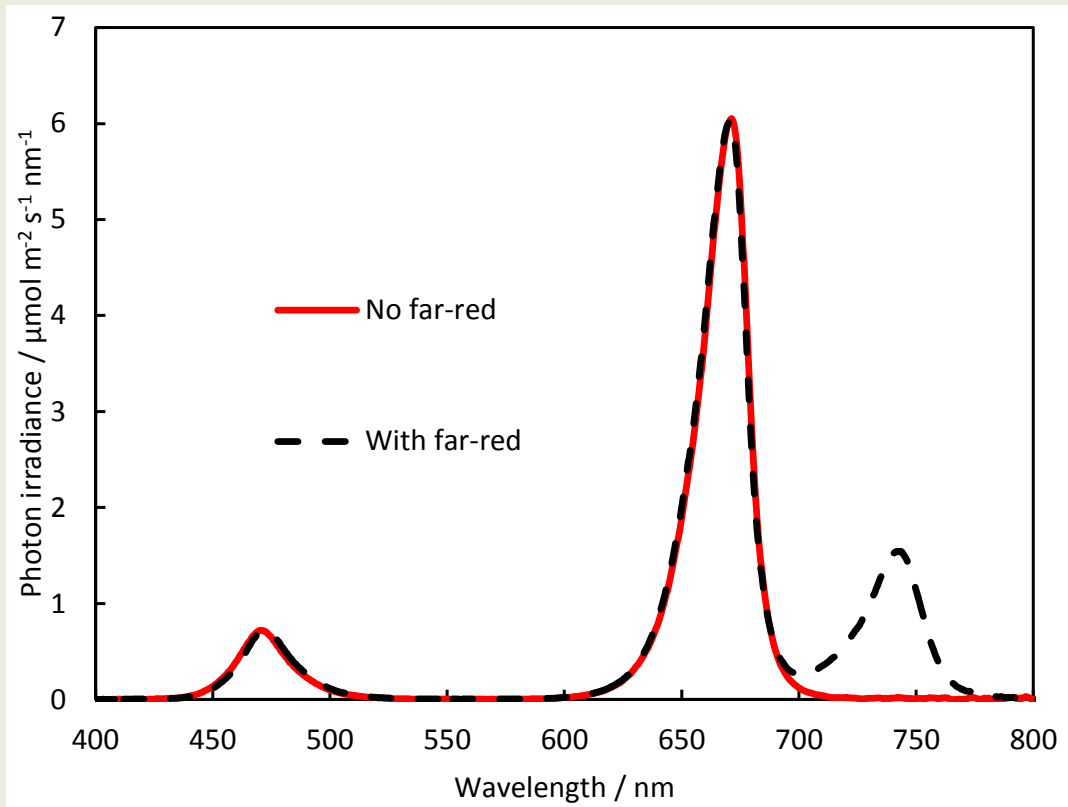
For this experiment, a small group of herb species was selected to assess the range of responses to far-red light. The aim of the experiment was to make qualitative assessments of the morphological responses experienced by the different species and to determine the similarities and differences between species.

Materials and methods

Mint, English Sage, Dill, English Thyme, and Bronze Fennel seeds were sown on oasis plugs and covered with plastic until germination. The germinated plants were thinned to one plant per plug. When the plants started to grow roots out of the oasis plugs, they were transplanted to 10 cm pre-wetted Grodan delta rockwool blocks. Plants were grown under Philips production modules. The light intensity was $186 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the spectral mixture was 15% blue and 85% red (Figure 1). For the far-red light treatment an additional $48 \mu\text{mol m}^{-2} \text{s}^{-1}$ of far-red light was applied. Day length was maintained at 16 hours for all treatments. The temperature in the facility was maintained at 21°C both day and night.

Plants were watered once per day using an automated ebb and flood irrigation system. The irrigation solution was a weak fertilizer mix with the EC maintained close to 2mS cm⁻¹ and a pH of 6.

Figure 1. Spectra of the two light treatments.



Results

Pepper Mint (*Menthe piperita*)

Mint plants grown without far-red light produced an average of 1.8 runners per plant. None of the plants grown in the presence of far-red produced runners (Figure 2). Far-red light greatly influenced the leaf morphology. In the absence of far-red light the leaves were flat, while the addition of far-red light caused the leaves to develop a curled/blistered morphology (Figure 2-C). Far-red light also increased the average internode lengths from 29.4 mm to 51.3 mm. There was large variation between individual plants; this was presumably due the genetic variety within the seed lot.

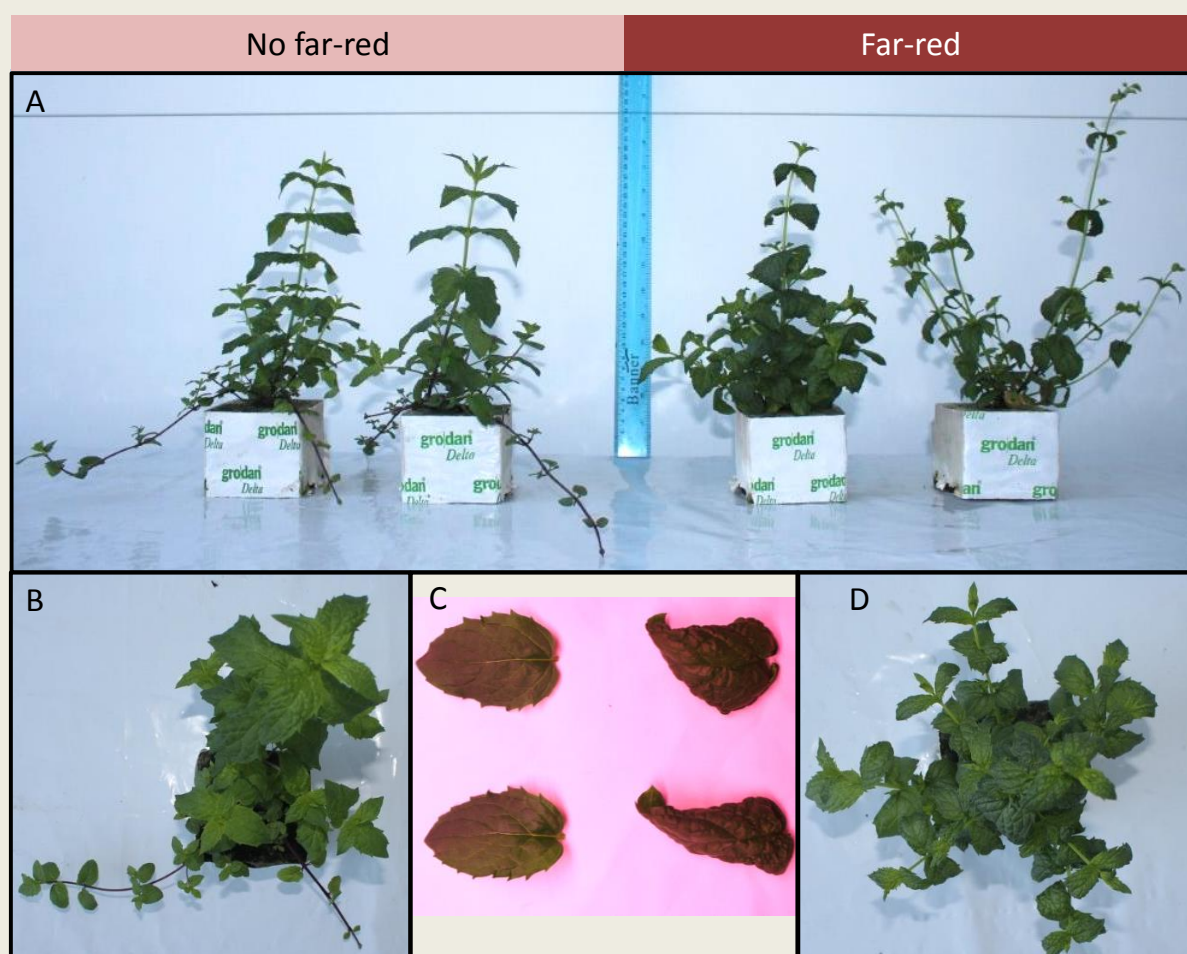


Figure 2. Morphology of pepper mint plants grown under red:blue LED (186 $\mu\text{mol m}^{-2} \text{s}^{-1}$: ~16% blue) lighting with and without additional far-red light. A) Comparison of plants grown without (two plants on left) and with (two plants on the right) far-red light. B) Plant grown without far-red viewed from above. C) Comparison of leaf morphology without far-red (left two leaves) and with far-red light (right two leaves). D) Plant grown with far-red light viewed from above.

Sage (*Salvia officinalis*)

Leaves of sage plants grown in the presence of far-red light demonstrated shade avoidance syndrome (Figure 3). This resulted in the normally elliptic leaves developing a narrower, more linear morphology and also a reduced size compared to those grown without far red. Far-red exposure did not induce changes in internode lengths (~ 25.5 mm). The smaller leaves of the far-red grown plants may have resulted in lower light interception and, therefore, a growth rate that resulted in the smaller stature of the far-red grown plants. The leaves of the plants grown under far-red light were a darker green in appearance compared to the no-far-red plants.

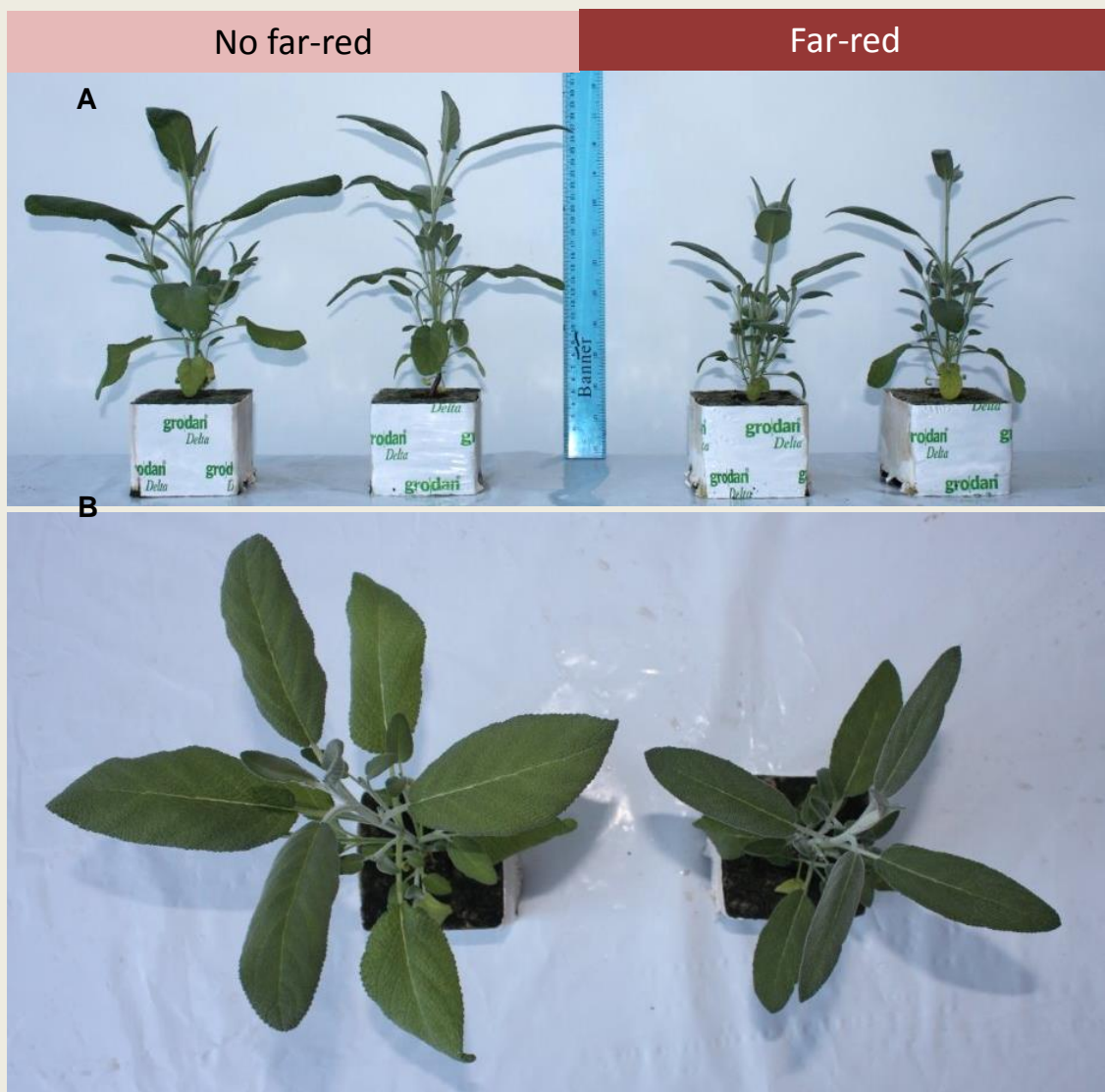


Figure 3. Morphology of sage plants grown under $186 \mu\text{mol m}^{-2} \text{s}^{-1}$ red:blue light with and without the addition of $48 \mu\text{mol m}^{-2} \text{s}^{-1}$ far-red light. **A)** Plants viewed from the side to demonstrate the differences in plant size. **B)** Plants viewed from above to demonstrate the differences in leaf shape and size.

Bronze Fennel (*Foeniculum vulgare* 'Purpureum')

On first inspection, Bronze Fennel plants grown in the two light treatments appeared to be similar in height and morphology (Figure 4). However, closer inspection indicated that, while the leaves were similar in length and gave the plants an appearance of similar stature, shoots were considerably shorter in the plants grown without far-red light (Figure 4 B and C). In plants grown without far-red light, the internodes were not visible as they were entirely enclosed in the sheathing bases of the petioles. Stem lengths were 4.7 times longer for the far-red treatment plants (172 mm long) than the no-far-red (36 mm long) plants. The morphology of the finely dissected leaves was similar between the two light treatments, but the far-red grown leaves displayed a slightly more feathery appearance.

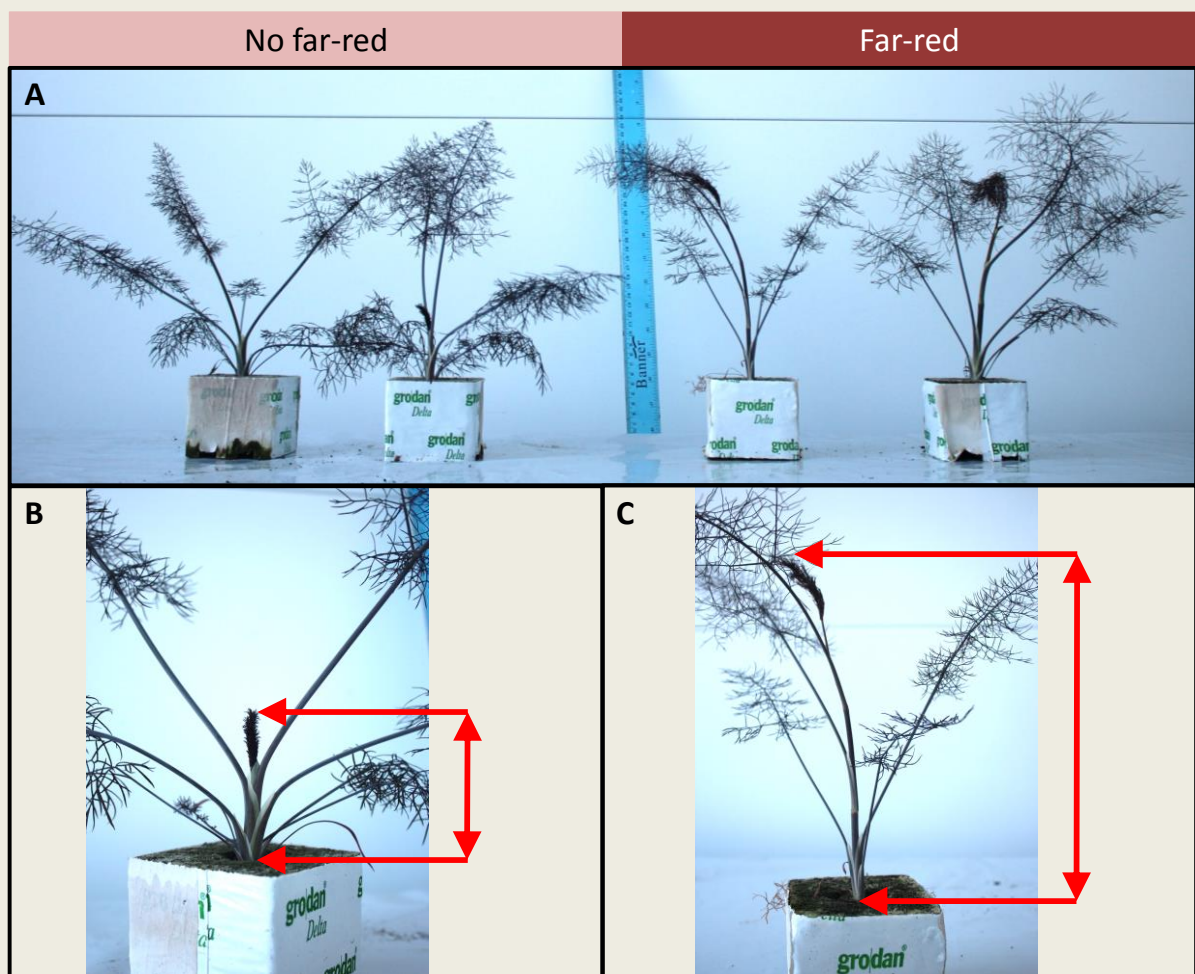


Figure 4. **A)** The appearance of bronze fennel plants grown in the absence (left two plants) and the presence (right hand two plants) of $48\mu\text{mol m}^{-2} \text{s}^{-1}$ of far-red light. **B** and **C** show close ups of plants highlighting the differences in shoot lengths between plants grown without (**B**) and with (**C**) far-red light. Arrows highlight the difference in shoot length between the two treatments. NOTE Scale of image B and C not equal.

Dill (*Anethum graveolens*)

Of the species examined, Dill exhibited the most pronounced responses to far-red light exposure. Plants grown in the presence of far-red light were three times taller than those grown without far-red, and far-red grown plants also flowered. Internode extension was responsible for much of the height increase. The internodes were not visible on the plants grown under no far-red light as they were hidden by the petiole bases, and the whole shoot was only 58 ± 7 mm long. By contrast, the internodes of the far-red grown plants were 125 ± 17 mm in length. The morphology of the filiform leaves was also strongly influenced by the light treatments, with both the arrangement and position of the leaf filaments being affected. Under far-red light, the leaves were shorter and had a more feathery appearance. The petiolules of the leaflets of the leaves were held at an angle of approximately 50° while they were held at approximately 26° in the absence of far-red. Leaves under the far-red light developed a bluish tinge that was absent in the no far-red leaves.

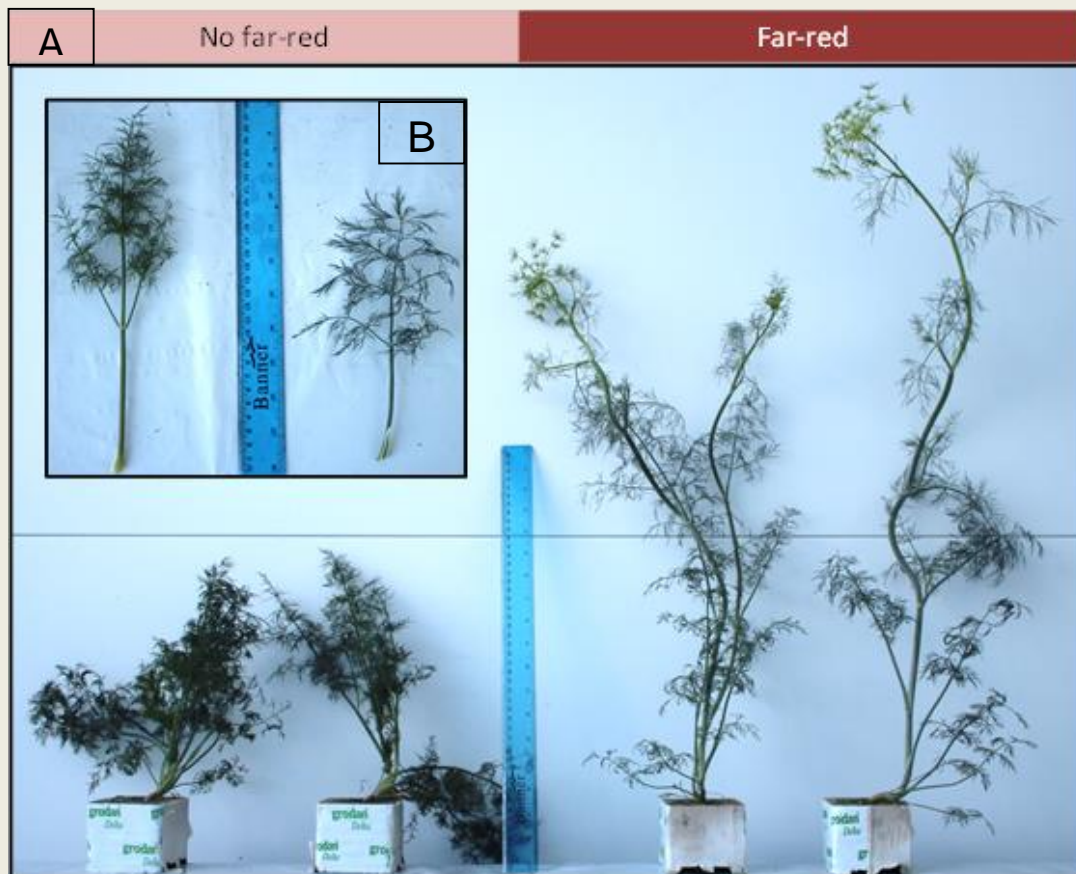


Figure 5. The influence of far-red light on the morphology and development of Dill plants. **A)** The two plants on the left side were grown without far-red light and the two plants on the right were grown with far-red light. **B)** The insert shows the morphology of the leaves grown without (left) and with (right) far-red light.

Thyme (*Thymus vulgaris*).

For thyme plants, the addition of far-red light caused a moderate increase in length of the internodes from 24 to 31 mm. The leaf morphology was strongly influenced by the addition of far-red light. The leaves were slightly smaller in the presence of far-red light (8.7 mm compared to 9.6 mm in length). Under far-red light the leaves were heavily curled, which greatly reduced the effective leaf area, see Figures 6B & 6C. The curling resulting from the far-red light caused the length to width ratio to increase from 1.8 to 3.

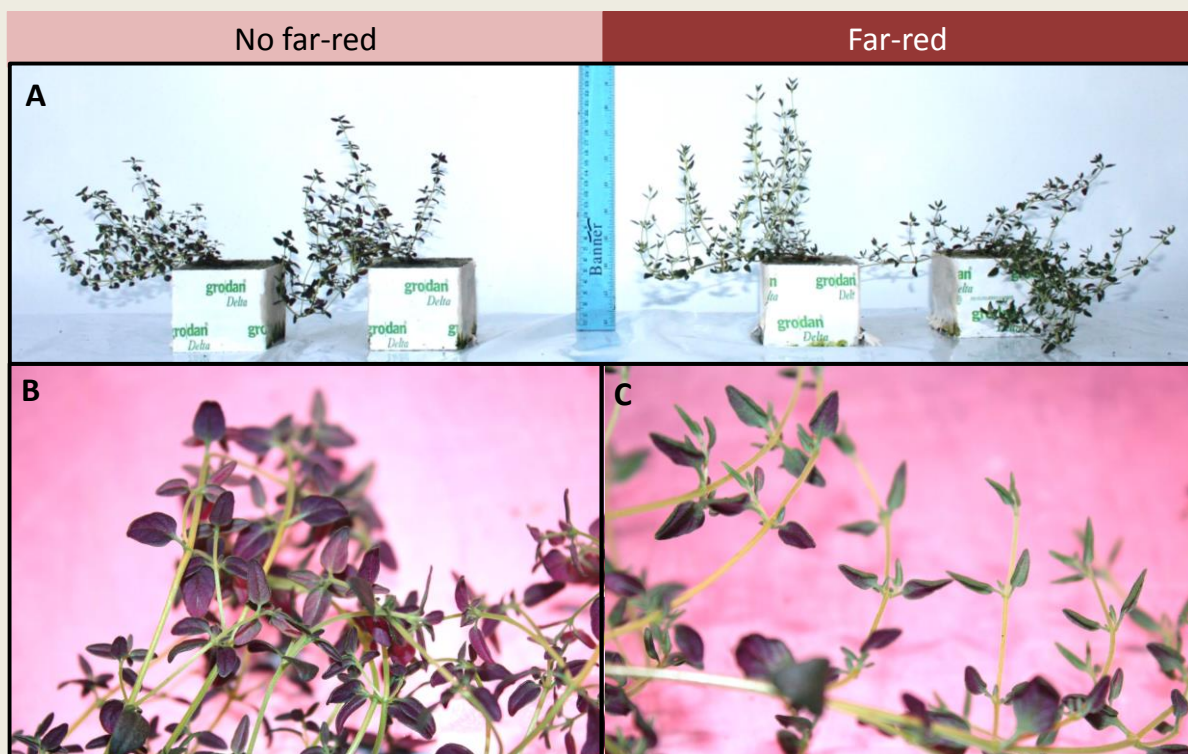


Figure 6. The influence of far-red light on the morphology of thyme plants. **A)** Side view of the plants grown without (left) and with (right) far-red light. The influence of far-red light on leaf morphology when viewed from below is shown in **B)** without far-red and **C)** (with far-red).

Discussion

All the plants grown in this trial responded to far-red light exposure by exhibiting one or more characteristics associated with plant growth in shaded environments. These responses are sometimes collectively called shade avoidance syndrome. Although these changes are aimed at helping the plant survive in lowlight conditions, and plants might therefore be expected to respond in similar ways, not all the plants in this study displayed the same adaptation magnitudes. Dill had the most extreme response to far-red light (large internode expansion, change in leaf morphology, and flower initiation). Sage was least responsive and only a change in leaf morphology was observed. The magnitude of the responses varied between the species but the response types seen were similar, with changes to internode length, leaf morphology, and flowering time observed. Of these, leaf morphology requires the most complex assessment in order to address factors such as leaf curling and the change in leaf shape. The metrics used to determine leaf morphology are dependent on species, leaf type, and the specific plant light response.

In this experiment, mint plants grown in far-red light produced no runners, while plants grown without far-red produced numerous runners. While this appears like a potential benefit of far-red on mint growth it should be noted that this was a small scale experiment and natural differences would be expected from genetic differences between seed. Larger-scale trials will be performed on Mint as part of AHDB Horticulture project CP125 and the influence of far-red on runner formation will be re-examined. Gaining an understanding of how plants respond to different light qualities provided by LEDs will be key to manipulating plant morphology and development to enable the production of good quality plants with maximum efficiency.

Chlorophyll is unable to absorb the far-red region of the light spectrum, and addition of this light does not, therefore, result in an increase in photosynthesis. Any responses resulting from the addition of far-red light will, therefore, be the result of altered morphology and development caused by the phytochromes. In full sun the red:far-red ratio of light is near to 1. In shaded low-light conditions, such as below a forest canopy, the red:far-red ratio will decrease as the shade increases. With artificial lighting, the red:far-red ratio can be altered independently of light intensity. In the case of LEDs, a range of light conditions can be created that are not likely to occur in nature. In these experiments, the red:far-red ratio used was either >200 for the no far-red treatment or 3.3 for the far-red light treatment. Plants grown with a red:far-red ratio of 3.3 demonstrated strong shade avoidance responses even though the red:far-red ratio was greater than that of full sunlight. This

demonstrates that red:far-red ratios alone cannot be used to assess plant light responses to different light sources. Several other factors must be considered. While the response of the phytochromes is generally discussed in relation to the red:far-red ratio, they are also able to sense and respond to light of all wavelengths between 300 and 750nm. This means that each spectrum of light will be sensed slightly differently.

Phytochromes are also sensitive to the intensity of the light and this allows plants to adjust their morphology to match all potential light environments. In nature, low far-red light environments are almost exclusively associated with low light conditions, but low light conditions are not always associated with low red:far-red ratios. Heavy cloud, shade induced by cliff faces or other non-plant structures, and low light levels encountered in winter months will result in lower intensities of light with a red:far-red ratio near to 1.

Using the equations of Sager et al (1988), two properties of phytochrome PhyB can be estimated: the phytochrome photostationary state (PSS) and the relative cycling rate (RCR). The PSS value is constant for any light spectra and is independent of the light intensity. The RCR value is influenced by both the spectrum and intensity of a light source and increases as light intensity increases. For the no-far-red light treatment used in this study, the PSS was 0.871 and the RCR was 0.1. Under far-red light conditions the PSS was lower, at 0.813, and the RCR higher, at 0.148. As a comparison, the same parameters were determined for sunlight with the same PAR light intensity ($186 \mu\text{mol m}^{-2} \text{s}^{-1}$). The PSS (0.719) and the RCR (0.082) were lower than in both LED light treatments. While these calculations provide additional information regarding the sensing mechanism of the phytochromes it remains unclear whether these data can be used to predict/compare the influence of different light spectra on plant morphology. The methods used to make these calculations will continue to be developed and evaluated during AHDB Horticulture project CP125 .

Conclusions

Far-red light had the least influence on Sage morphology and the greatest influence on Dill, which was the only species to flower during this experiment.

Plant responses to far-red light were diverse in magnitude but the effects could be assessed with three major morphological assessments: increased stem elongation, altered leaf morphology, and flowering time.

Leaves from different species responded differently to the addition of far-red light. Some leaves curled (Mint, Thyme) and some became narrower (Sage). The filiform leaves of Dill and Fennel become more feathery in response to far red light.

The red:far-red ratio of light cannot alone be used to predict plant responses to lights with different spectra. Additional methods have been developed to assess different aspects of the phytochrome light sensing properties. However, more experimentation is required to determine how useful these methods will be for assessing plant light responses.

Knowledge and Technology Transfer

PRESENTED AT

South Holland Growers Club Meeting, Spalding, Lincolnshire, February 2014.

Teehee Lane Allotment Society, Bebington, Merseyside, February 2014.

UV4Growth Odense, Denmark, March 2014.

York Festival of Ideas, York, June 2014

Vertical Growing and Urban Agriculture, Nottingham, September, 2014

PUBLICATIONS

Authored by Dr Davis

Coloured judgement. AHDB Grower, February 2014, 200:12-13.

Glossary

PGR = Plant growth regulator

PAR = Photosynthetically active radiation (400-700nm) measured in units of $\mu\text{mol m}^{-2} \text{s}^{-1}$.

HPS = High pressure sodium lamp.

HID = High intensity discharge lamp.

Petiolule = The stem of a leaflet from a compound leaf.

Petiole = The stem of a leaf.

Internode = The section of stem between two leaves or nodes.

Photoreceptors = An organic molecule that can absorb light of a specific colour or colours and use this light to initiate a signal cascade that results in a physiological and or morphological change of the plant.

Phytochromes = A family of photoreceptors that enable plants to sense red and far-red light.

Cryptochromes = A family of photoreceptors that enable plants to sense blue and UVa light.

Phototropins = A family of photoreceptors that enable plants to sense blue and UVa light.

PSS = Phytochrome photostationary state.

RCR = Relative cycling rate.

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Appendices

Appendix I - Analysis of contacts made during the first two years of this fellowship.

Links have been developed with a number of individuals and companies. In this section, contacts are indicated for different sectors of the horticulture industry. Company counts rather than individual counts are used in order to avoid bias where contacts were developed with multiple individuals from one organisation

GROWERS

Protected Edibles = 17

Tomato = 6

Herbs = 6

Salads = 4

Peppers = 1

Protected Ornamentals = 17

Bedding= 11

Cut flowers = 2

Bulbs and stocks = 4

HNS = 1

Field Vegetables = 3

Soft fruit = 2

Propagators = 2

More emphasis will be given to HNS in year three

HORTICULTURE SUPPLIERS AND SUPPORT

Seed companies = 5

Substrate manufactures = 5

Consultants = 5

Pesticides companies = 2

Spectral filters = 3

Glasshouse/poly tunnel builders = 3

TECHNOLOGY COMPANIES

Lighting companies = 9

Electronics and sensor companies = 7

FOOD PROCESSORS AND RETAILERS

Retailers = 3

Processors = 4

FUNDING AND RESEARCH

Nongovernmental organisations = 4

Funding agencies = 4

Universities

UK = 7

Europe = 10

USA and Canada = 6

Asia = 6