

Project title: Understanding crop and pest responses to LED lighting to maximise horticultural crop quality and reduce the use of PGRs

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

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GROWER SUMMARY

Headlines

- Spectral manipulation using LEDs can be used to control plant morphology and flowering time.
- LED lighting can provide the optimal conditions for rooting cuttings.
- Lighting mother stock plants during the winter months increases cutting quality and strike rates.
- Early results indicate that light quality can be selected to maximise plant resistance to pests.
- Biocontrol agents can successfully identify pests under LED lighting in controlled conditions.

Background

The experiments reported here are arranged in three work packages.

Work package 1 - General agronomy under LED lighting

This work package will examine the general agronomic practices required for plant production under LED lighting. One of the major benefits of LED lighting is their low energy consumption compared to conventional lighting systems. Their robust nature and ability to rapidly turn on and off also provides the possibility of further reducing energy consumption by either creating mobile light rigs that move over the crops at regular intervals or strobing the light to reduce energy consumption. Both these techniques can lower energy consumption, but this comes at the cost of a lower daily light integral (DLI). The results from year one demonstrated that mobile and strobe lighting systems designed to reduce capital and electrical running costs had a negative impact on plant performance and quality. This was caused by the combined effect of a reduced DLI and reduced plant light use efficiency. The work reported here (year 2) will focus on furthering our understanding of the influence of constant light intensity on plant quality, growth rate and running costs. The growth of Petunias, Pansies and Lettuce were examined in this work package.

Work package 2 - Influence of light quality on crops

The experiments in work package 2 examine the responses of plants to different light spectra with the aim of improving our understanding of the diversity of plant responses to light and to help commercial implementation of LED technologies. WP 2 is divided into subsections examining different aspects of light quality on plant morphology. This report contains results from four subsections of WP 2:

WP 2.1b: Influence of red : blue ratio on plant growth.

WP 2.1c: Influence of red : far-red ratio on plant growth.

WP 2.1d: Red : blue : far-red light combinations.

WP 2.3: Improving cutting propagation.

Several species were examined (Petunia, Pansy, Lettuce, Santolina, Clematis, Iberis). Where appropriate, plants of the same species were grown simultaneously in multiple work packages. The results are reported in groups based on work packages.

Work package 3 - Light quality and its influence on pests

During the first year we examined the use of different colours of sticky traps on pest trapping efficiency. In this report we examine the influence of light quality on aphid and spider mite performance cultured on Lettuce, Verbena and Cucumber plants grown under different light treatments. The effectiveness of biocontrol agents was also examined under different light treatments.

Summary

WP 1.2 - Energy saving and daily light integral

In general increasing the light intensity resulted in faster growth, more robust and compact plants with earlier flowering. However, providing too much light leads to plant stress especially at the seedling stage and increased installation and running costs. It is important to achieve a balance between providing enough light for good quality plant material while minimising the costs to maintain a strong economic basis for production.

In these experiments $200\mu\text{mol m}^{-2} \text{s}^{-1}$ was enough light to produce good quality Pansies, Petunias (Figure GS1) and Lettuce plants (propagation stage). In all cases providing less light resulted in slower growth and lower quality plants and did not result in an energy saving when the additional time required to produce crops was included in the analysis.

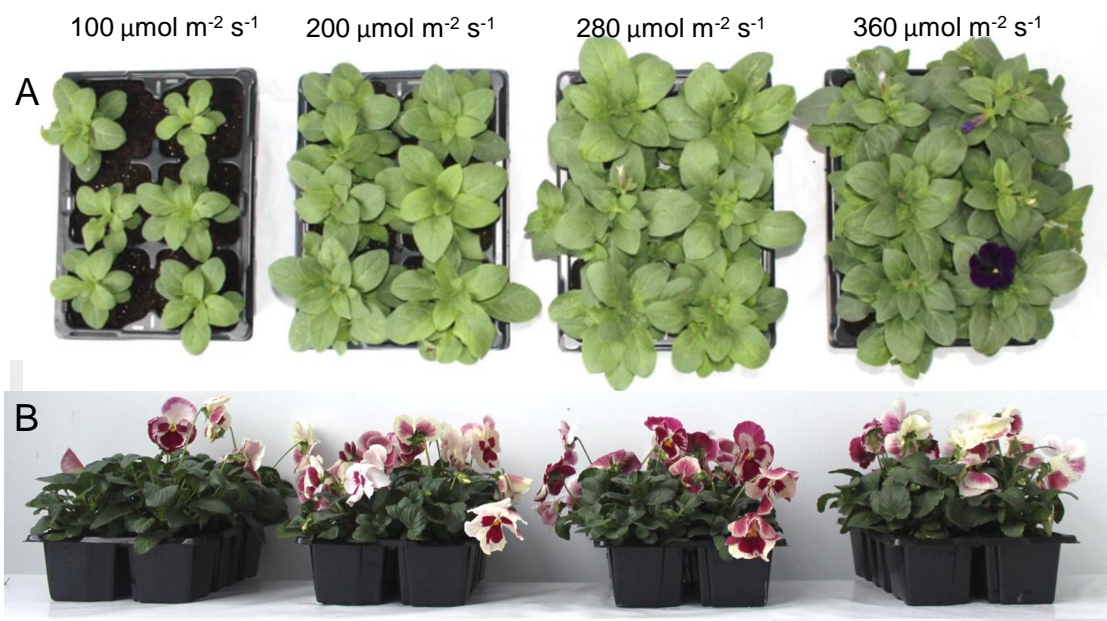


Figure GS1. The influence of different intensities of light in growth of **A)** Petunias 26 days after sowing and **B)** Pansies 74 days after sowing.

Higher light intensities (280 and 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$) resulted in faster production and higher quality Petunias and Lettuce plants with thicker more robust leaves. Petunia plants grown under the highest intensity flowered five weeks after sowing. In contrast Pansies grown under the highest light intensity flowered only marginally quicker than those grown under 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and were very compact. Also the Pansy seedlings grown under the highest light intensity performed poorly and lower numbers of good quality plug plants were produced.

In summary a light treatment with an intensity of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provides a good starting point for plant growth under LED lighting. Increasing the light intensity can help produce more robust plants and may hasten flowering. The optimum light intensity will differ between plant species (sun plants will benefit from higher light intensities than shade plants) and based on the desired properties of the final product.

WP 2.1b - Influence of red / blue ratio on plant growth

The most energy efficient LED lighting systems contain predominantly red and blue LEDs (see AHDB CP 139). Red and blue light can efficiently drive plant photosynthesis and control morphology. In the year one trials we demonstrated how different mixtures of red and blue light influenced plant growth and morphology. Plants grown under 100% red or 100% blue light were etiolated and had poor overall quality. Growth rates were greatest in

plants grown under red/blue mixtures containing 11-15% blue light. The most compact plants were observed under light containing about 60% blue light. These data demonstrate the potential to use light treatments to replace the use of plant growth regulators. During this year we have repeated the experiments on Lettuce three times to create a robust data set that can withstand more detailed analysis (to be completed as part of the parallel CP 085 Fellowship programme) and to demonstrate the consistency of plant quality grown under constant light conditions. The data (Figure GS2) demonstrate the contrasting influence of light quality on biomass and morphology. The data on leaf size from the three experiments was highly reproducible.

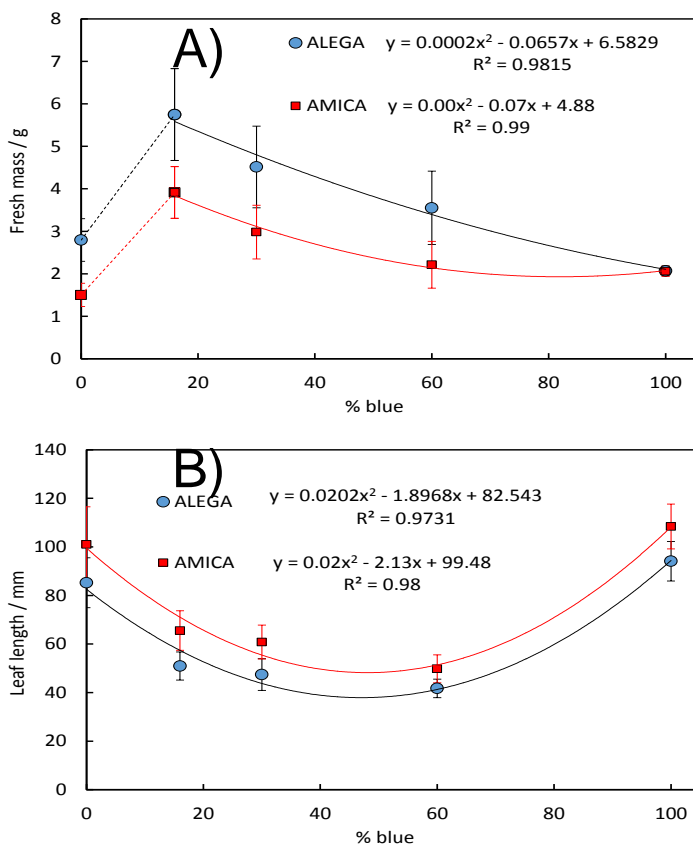


Figure GS2. Influence of blue light percentage on **A)** the shoot biomass and **B)** leaf length of two Lettuce varieties (Amica, a summer variety and Alega, a winter variety).

The influence of red:blue light spectra on Petunia flower development and flower size was also investigated in more detail. Flowers grown under light with 60% blue light were observed to open two days faster than flowers grown under 6% blue light (Figure GS3). Flowers that opened more rapidly were also found to remain open for a longer period, resulting in greater numbers of open flowers per plant.

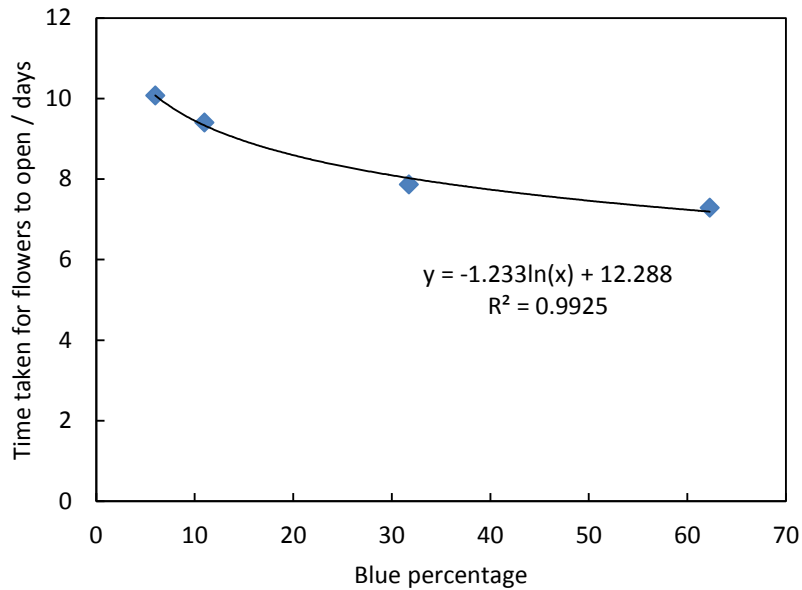


Figure GS3. Time taken for Petunia flowers to open when exposed to light treatments with different percentages of blue light.

WP 2.1c - Influence of red / far-red ratio on plant growth

The results generated in WP 2.1c in year one examined how different doses of far-red light influenced the growth of eight species (Basil, Sage, Cucumber, Lettuce, Petunia, Pelargonium, Pansy, Begonia). In general far-red caused plants to grow taller and flower though the magnitude of the responses differed considerably between species: Basil plants showed small responses to far-red light while Cucumber exhibited large responses. The experiments in WP 2.1c reported here have focused on generating a replicated data set in Lettuce. Far-red treatments were found to have a strong influence on leaf size. Altering the far-red light dose could be a useful method for manipulating plant morphology and appearance to enable crops to be grown to match the needs/preferences of end users.

WP 2.1d - Influence of high blue and far-red light treatments

The data generated in WP 2.1b and WP 2.1c described the benefits of red:blue (compact plants) and red:far-red (early flower) spectral manipulation. However, they also highlight the limitations of these manipulations. Red:blue manipulation can lead to slower growth and delayed flowering. Red:far-red manipulation can result in reduced pigmentation and plant stretching. This work package examines the potential to combine high blue and far-red treatments to produce compact plants that flower early. Petunia, Pansy and Lettuce were grown under eight light treatments comprising two red:blue mixtures (30:70 B:R and 60:40 B:R) each with four different intensities of far-red light (0, 11, 20 and 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

The plant responses to red:blue:far-red combinations were consistent with the data from the other work packages. 30% and 60 % blue produced compact plants and far-red caused plants to stretch and induced earlier and more extensive flowering (Figure GS4). High-blue light treatments were unable to prevent the plant stretching caused by far-red light treatments. However, low intensity far-red treatments combined with high blue treatments were able to produce early flowering plants with less etiolation than would be the case for lower blue percentage treatments. In these experiments the best quality plants were produced under the 30% light blue treatments and the addition of far-red light advanced flowering by up to two weeks. The addition of greater than $11 \mu\text{mol m}^{-2} \text{s}^{-1}$ of far-red light had deleterious effects on morphology. Further light recipe development should focus on lower intensities of far-red light to identify a treatment that can induce flowering with minimal impact on morphology or short term far-red treatments that induce flowering but have little influence on morphology.

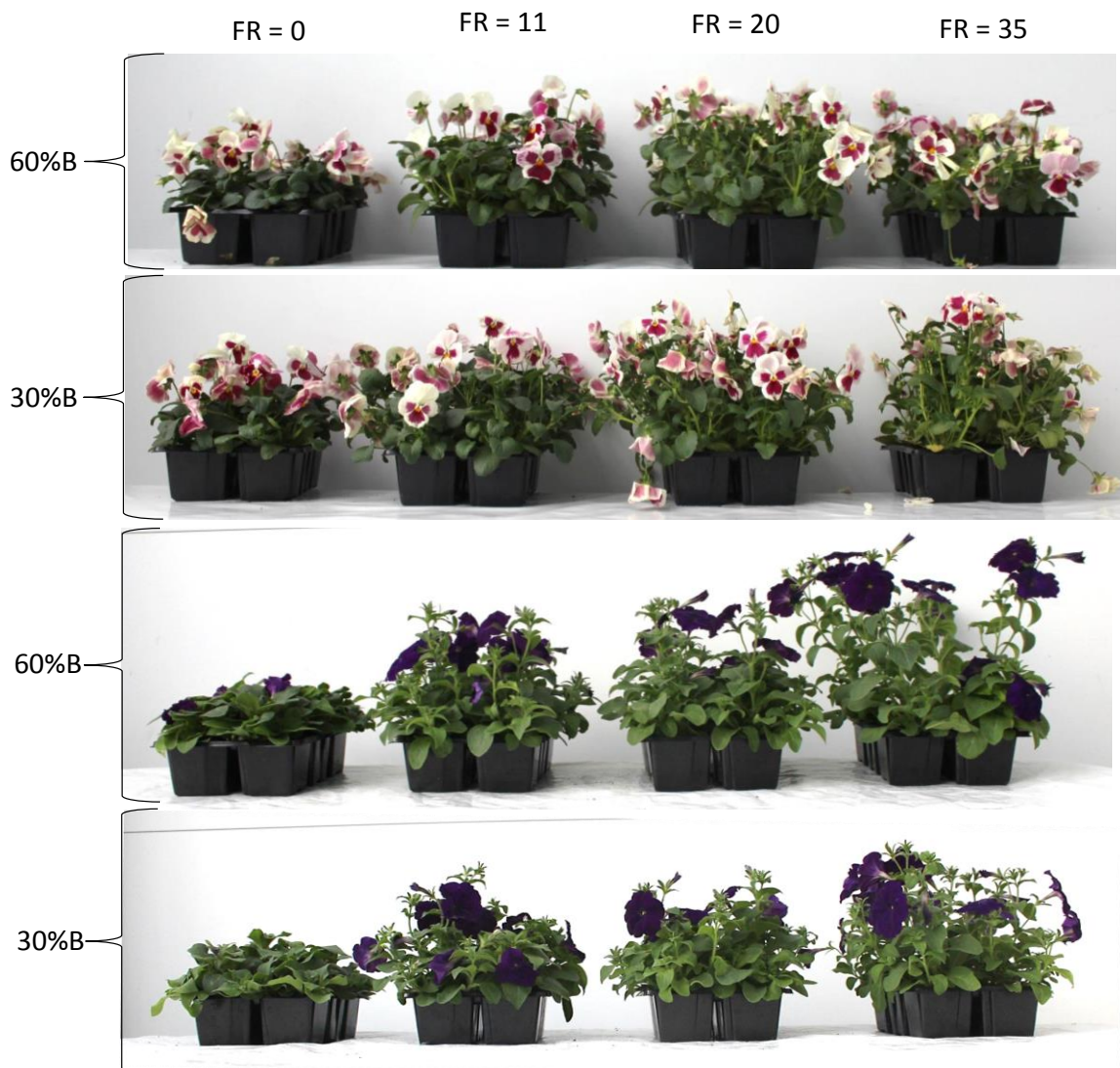


Figure GS4. Influence of red, blue and far-red light combinations on the morphology and flowering of Pansies and Petunias.

WP 2.3 - Improving HNS Propagation

Spectral manipulation of liners can improve strike rates. Reducing the amount of blue light in the spectrum, while providing sufficient light to maintain plant health and vigour, reduces cutting dehydration and greatly improves survival. This is particularly important during the first week after sticking. The light spectrum also influenced the speed and vigour with which roots developed. Maximum speed and percentage of rooting was achieved in treatments with little or no blue light (Figure GS5). 100% red, 90% red + 10% blue and a red:white treatment containing 9% blue resulted in the best rooting for Santolina 'Lemon Fizz', Clematis 'The President' and Iberis 'Absolutely Amethyst' cuttings. For Clematis, tip cuttings strike rates were on average 13% higher than for nodal cuttings but the influence of light treatments was similar on both types of cutting. Far-red light had a negative influence on liner quality and rooting success. The health of cuttings exposed to far-red light deteriorated more rapidly than those not exposed to far-red light. The amount of light provided to cuttings is also important for both cutting success and system economics. Too much light will stress the cuttings while too little light will weaken cuttings reducing the resources available for root growth. Santolina and Clematis nodal cuttings rooted equally well in 36 and 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light, other species may benefit from higher or lower intensities.

Lighting mother stock plants through the winter months was shown to greatly influence cutting strike rate (Figure GS6). In this experiment the Santolina mother stock plants lit with 51 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of LED light produced cuttings with greater strike rates (70% rooted) than unlit (50% rooted) plants. Iberis cuttings also benefited from the supplemental light treatments though it was rooting speed rather than absolute strike rates that were improved. Ensuring light treatments are correct is also very important as incorrect lighting can reduce cutting survival. Santolina mother stock plants illuminated with low intensity night break lighting produced weaker cuttings with lower strike rates (~30% rooted) than unlit plants. It is thought that in this case the night-break lighting forced mother stock plants to grow, but the low natural light levels provided insufficient resources to maintain vigour resulting in weaker cutting material.

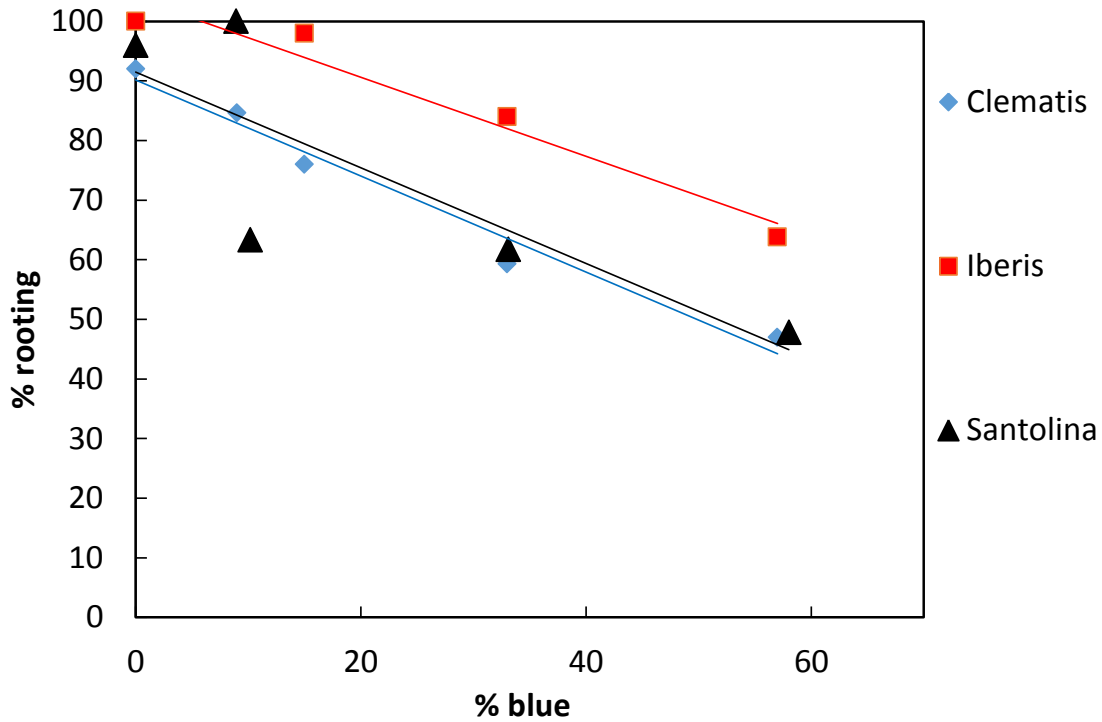


Figure GS5. The influence of blue light percentage of post-excision light treatments on Clematis (tip cuttings), Iberis and Santolina cutting strike rates.

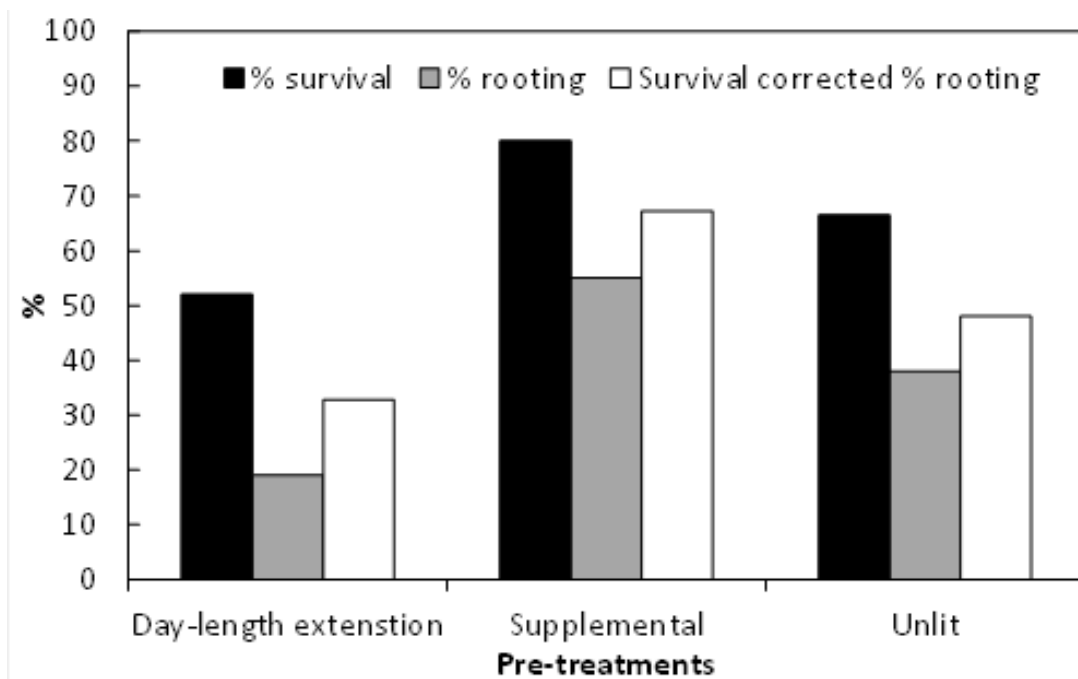


Figure GS6. The influence of pre-excision light treatment provided to the mother stock plants on the percentage of survival and rooting of the Santolina cuttings.

WP 3.3a - Determining the effects of plant physiology on Aphid development

During these experiments we encountered a number of challenges associated with culturing insects on plants grown under the LED lighting systems. Many of these challenges are thought to be caused by the light environment either via direct effects on the insects or indirectly via the plant light responses. While challenging from the perspective of performing experiments these difficulties are encouraging as they indicate that pest species would be expected to perform less well on LED grown plants and optimised lighting may provide some level of pest control.

When peach aphids were grown on Lettuce plants grown under different red:blue ratios aphid mortality was found to be significantly higher on plants grown under 60% blue light. This was thought to be associated with the aphids being unable to feed due to the physical characteristic of the Lettuce leaves (small compact leaves). When melon aphids were grown on Verbena (Figure GS7) aphids grew significantly less well on plants grown under 100% red light and significantly better under white light than on plants grown under red:blue mixtures. The leaves of Verbena plants have a curled morphology when grown under 100% red light and this may have influenced feeding. The white LED lights used generally produced a softer plant than red:blue light mixtures and this may have resulted in better aphid performance.

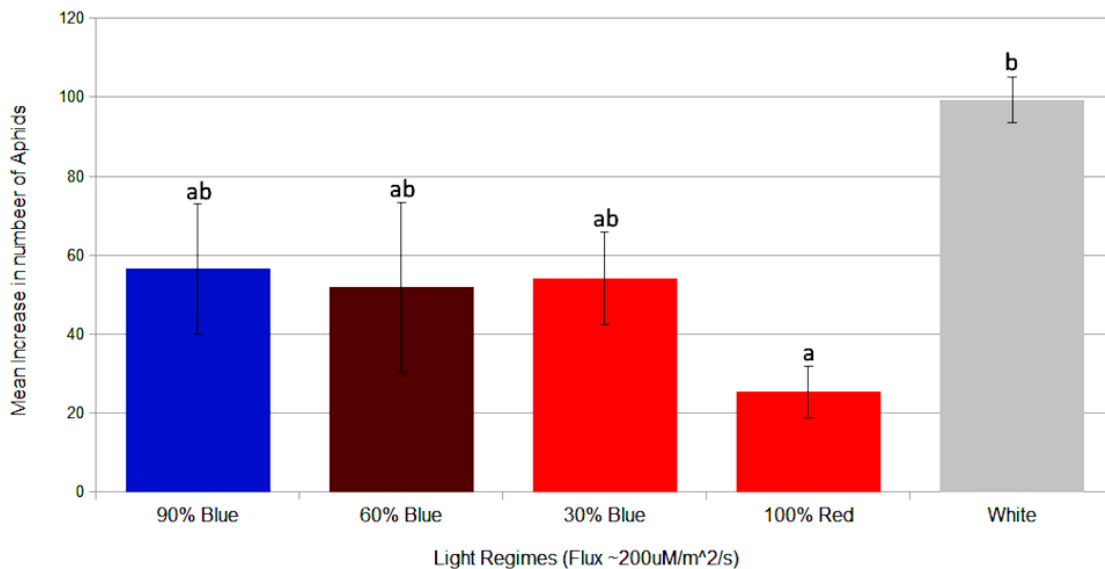


Figure GS7. Influence of light quality on melon aphid population growth on Verbena over a 10 day period. Error bars are standard error at n=6, and letters indicate significance groupings according to a TukeyHSD test ($p < 0.05$).

WP 3.3b - Determining the effects of plant physiology on spider mite development

Spider mites were cultured on Cucumber plants grown under a range of red blue spectra as well as a white light mixture. Spider mite population growth was slowest under a light treatment containing 30% blue and 70% red light (Figure GS8). The populations grew most rapidly under 100% red and 90% blue,10% red light. This suggests that both red and blue light plant responses are involved in plant defence against spider mites.

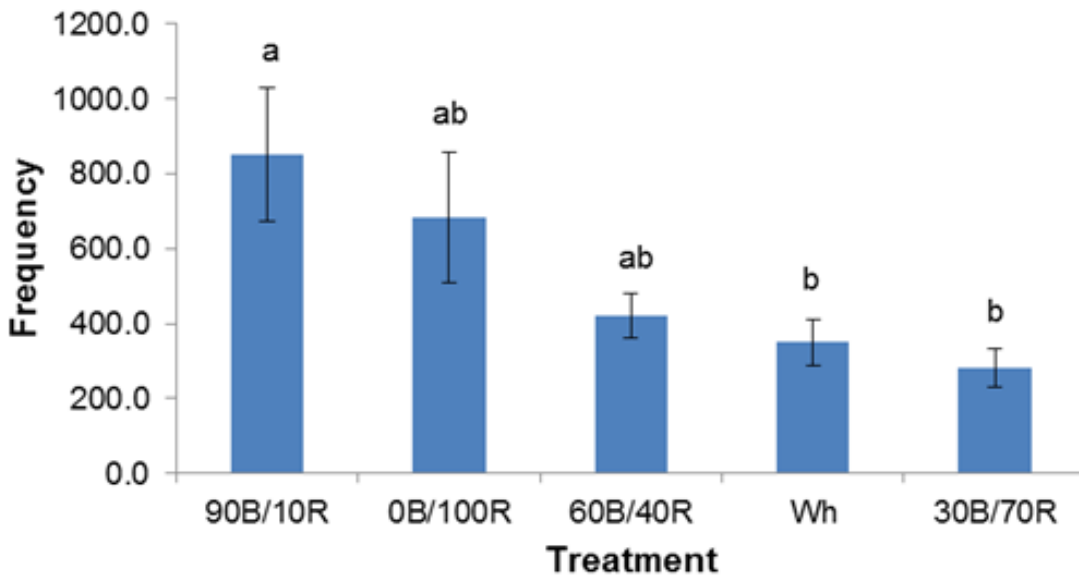


Figure GS8. Numbers of spider mite on Cucumber plants grown under light treatments with different red:blue mixtures. Error bars represent the standard error of the mean and Tukey's test significances. Letters above bars correspond to the results of the Tukey tests, where means not sharing a common letter are significantly different.

WP 3.4a - Parasitoid wasp activity

Biocontrol agents play a major part in pest and disease management programs and are likely to become increasingly important as regulations reduce the availability of pesticides. In non-controlled conditions our early attempts at using parasitic wasps in the LED4CROPS facility have resulted in no parasitism. It was unclear whether these attempts failed due to releasing too few predators or due the inability of the predators to effectively identify pests. In these experiments we investigated how effective parasitoid wasps (*Aphidius matricariae*) were as aphid biocontrol agents under red:blue light mixtures. When confined to the plants infested with aphids, in plastic bags, the parasitic wasps were able to identify and parasitize aphids. The greatest amount of parasitism was observed in treatments containing 30% blue light (Figure GS9). There were large differences in the amount of parasitism between the replicate trials that are thought to be associated with insect age.

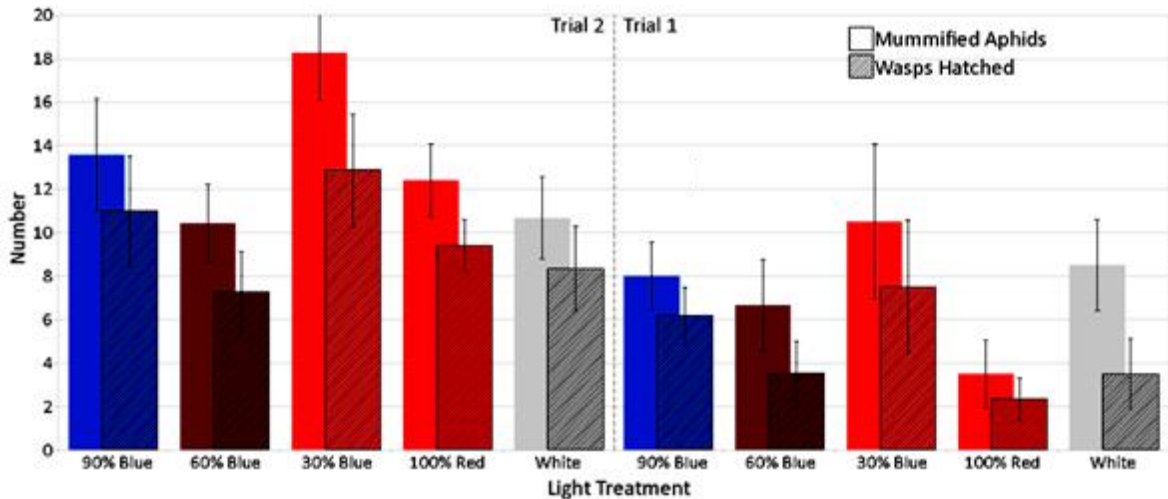


Figure GS9. Mean number of mummified aphids (solid bars) 10 days after exposure to two female wasps for 24 hours, and the number of new wasps (shaded bars) that had hatched from those mummies after a further 10 days. Data from two replicate trials are presented. Error bars are standard error at n=6 (Trial 1) and n=7 (Trial 2).

We also investigated if the light treatments influenced the activity of wasps during the illuminated period as differences in activity could be influencing rates of parasitism between treatments. Wasp activity was correlated with spectral quality of light with greater activity occurring under treatments with more blue light (Figure GS10). This is probably associated with the greater visual sensitivity to blue than red light.

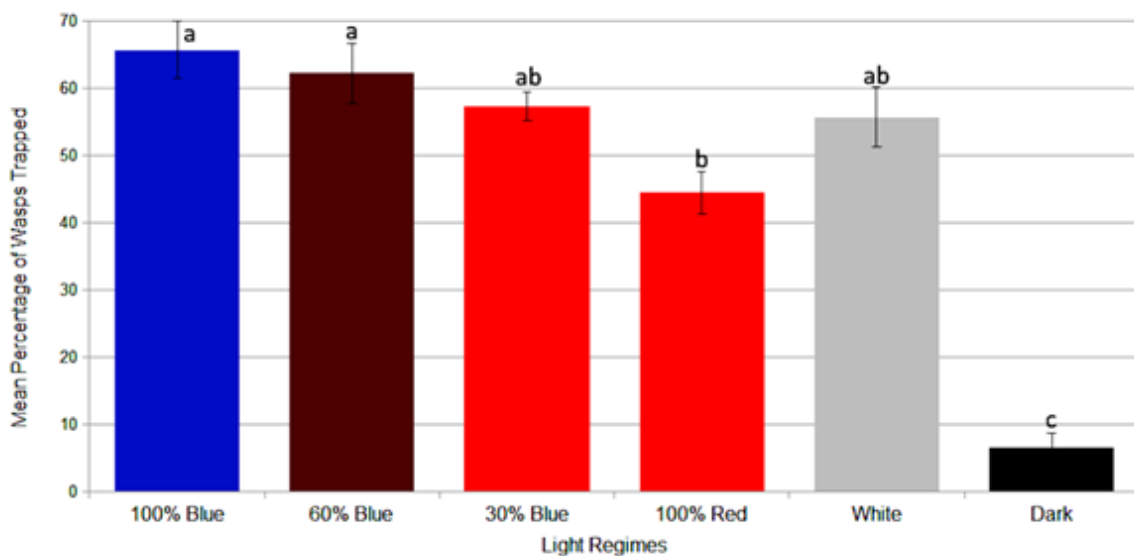


Figure GS10. Mean percentage of wasps caught in a colourless sticky trap only accessible by flight during a single 24 hour day/night period under different ratios of red and blue LED illumination, and in dark and white light. Error bars are standard error at n=8, and letters indicate significance groupings according to a TukeyHSD test (p<0.05).

Research Highlights

- Plant growth rate and quality increases with light intensity but maximum energy efficiencies were achieved at $\sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$.
- Plant growth rate was greatest under light mixtures containing $\sim 10\%$ blue light.
- Maximum growth regulation was achieved under light mixtures containing between 30 and 60% blue light.
- Far-red light can advance flowering by up to two weeks but has a negative impact on morphology.
- Careful selection of the blue light percentage and far-red intensity can be used to produce high quality plants with rapid flowering.
- Cutting strike rates were best in plants propagated under 100% red light.
- Lighting mother stock plants greatly improves cutting quality and strike rate.
- Pest performance appears to be inhibited by the LED light regimes used in these trials.
- The influence of light quality on pest performance differs between different pest-host combinations.
- Light quality also influences the effectiveness of biocontrol agents.

Financial Benefits

Advances in LED technology continue to improve LED energy efficiency with the newest systems achieving efficiencies of 2.8 mol J^{-1} , a 45% energy saving compared with 600W HPS lamps which have an efficiency of 1.92 mol J^{-1} . The economic benefits associated with these significant energy savings could become considerable as energy prices increase with time. The ongoing research and development in to design of LED lighting systems will be expected to keep the costs of LED units relatively high compared to HPS systems for some years, however, improved energy efficiencies will reduce installation costs as fewer units will be required to provide the same intensity of light.

The results in this report demonstrate that the ability to control the light spectrum with LEDs creates the potential to produce high quality plants and reduce the need for plant growth regulators. Cutting strike rates can be greatly improved by illuminating cuttings with spectra containing low blue and high red light proportions. Lighting mother stock plants through the winter months has the potential to further improve strike rates by maximising the quality of cutting material. These benefits potentially have greater impact on business economics than electrical energy savings.

The results from these trials provide the first steps in defining optimal lighting conditions for a range of crops. This information will help growers considering investing in LED installations and help ensure that light installations have the appropriate spectra for their

crops. For certain crops there may not currently be a complete LED solution available. However, these data could help LED manufactures design lighting systems that meet the needs of different crops.

Action Points

To make use of most of the data generated in this report, growers would need to invest in LED lighting systems. Costs of lights and economic analysis of the benefits are beyond the scope of this report and will be unique to each business. However, these results outline the benefits provided by different regions of the light spectrum and how light intensity influences plant quality. These results will provide a baseline from which growers can begin to develop their own light treatments while performing small scale trials. It is recommended that small onsite trials are carried out before large scale investments are made. This is for two reasons 1) to ensure the light treatments are appropriate for the specific varieties being grown and 2) to help growers develop the appropriate crop management strategies (it is expected that LED lighting systems will result in altered crop water and heating requirements). At latter stages in this project more information will be provided to help growers learn how to manipulate crops with LED lighting.

The cutting rooting experiments indicate that light spectra have a large influence on strike rates. LED lighting systems can be used to greatly improve rooting efficiency of cuttings directly or indirectly if mother stock plants are lit. Propagation requires relatively low intensities of light so installation and running costs would be proportionally lower than for crop growth. If the installation of lights are deemed too expensive similar results may be achievable by using spectral filters that remove the majority of blue light.

For growers interested in using LED lighting we have roughly outlined the steps that should be taken to ensure a successful installation.

1. Identify the desired outcome of a lighting system *i.e.* improved crop quality, increased yield or reduced energy consumption.
2. Determine the lighting regimes required to achieve these goals and consider whether LEDs are required or if spectral filters can be used.
3. Conduct small scale trials to examine crop performance and learn how management strategies will need to be revised.
4. It is important to have accurate measurements of the light environment within a crop production area when performing lighting trials. LED lighting systems should not be measured using Lux meters. The best type of sensor for measuring LED lighting for crop production would be a PAR meter which measures the light that can be used by

plants for photosynthesis and makes measurements in units of $\mu\text{mol m}^{-2} \text{s}^{-1}$ – for more information see the AHDB Horticulture technical guide ‘Lighting: The principles’.

5. Use the trial results to determine the economics of an LED lit production system.

SCIENCE SECTION

Introduction

Maintaining high plant quality is of vital importance in commercial horticulture. In most cases, compact plants are most desirable and any etiolation (which often occurs in low light conditions encountered in the winter months) reduces plant quality and may affect sales. In the ornamental sector plant morphology is often controlled with plant growth regulators (PGRs) but changes to pesticide regulations may lead to reduced PGR availability. In addition, PGRs are usually unavailable for use in the protected edibles sectors, so alternative management strategies that span a range of crop sectors are desirable.

Plants use light through the process of photosynthesis to fix the carbohydrate that powers growth. The amount of light that plants receive influences the rate at which plants can grow but also has an influence on plant morphology and development. Plants possess several light sensitive compounds called photoreceptors which they use to sense their light environment. These photoreceptors are involved in regulating all aspects of plant biology and they enable plants to alter their morphology to match their environment. Photoreceptors also determine the optimal timing for the transition from vegetative to reproductive growth. Plant light responses have evolved to maximise the chance of survival and reproductive success, but not all these responses are desirable in commercial horticulture. Photoreceptors act to suppress etiolation, which occurs in low or poor-quality light conditions. As these responses are light regulated, spectral manipulation using LEDs, or potentially spectral filters, may allow control of plant morphology without the need for PGRs. Modification of the light spectrum in plant production facilities, using LEDs or spectral filters, will enable the manipulation of plants to either inhibit undesirable responses such as stretching (etiolation) or enhance desirable responses such as increased leaf or flower pigmentation. Successful implementation of spectral manipulation will allow improved plant quality and consistency while also reducing the need for plant growth regulators.

The photoreceptors can be roughly grouped according to the colour of light to which they are most sensitive: UVB light, blue light, or red and far-red light (Figure 1). UVB responses are regulated by the UVR8 photoreceptor. UVB light is highly damaging to plants and so these photoreceptors are very responsive to low intensities. UVB / UVR8 causes plants to increase their pigmentation, reduce stem extension, and increase the robustness of plant tissues. Blue light is sensed by several families of photoreceptor. The cryptochromes and phototropins regulate a wide range of blue responses (including plant height, pigmentation, leaf morphology, phototropism, stomatal opening, and circadian rhythms) that are relevant to producing high quality plants. The red and far-red responses are regulated by a family of

photoreceptors called the phytochromes. These are perhaps the most researched photoreceptors, and regulate many plant light responses (including plant height, pigmentation, leaf shape, circadian rhythms, induction of flowering, and day-length sensitivity). Many plant responses, for example plant height, are regulated by multiple photoreceptors and full control of these responses is likely to require developing light treatments that provide the correct balance of each colour. As multiple responses are under the control of light, light treatments designed to influence one aspect of plant quality may have negative impacts on other aspects. For example, light treatments designed to enhance leaf pigmentation may result in slow growth and delayed flowering. The experiments reported here are designed to increase our understanding regarding how several plant species relevant to the protected edible, protected ornamental, and hardy nursery stock sectors respond to different light qualities. This improved understanding will help the development of light regimes optimised for production of high quality plants with the characteristics required.

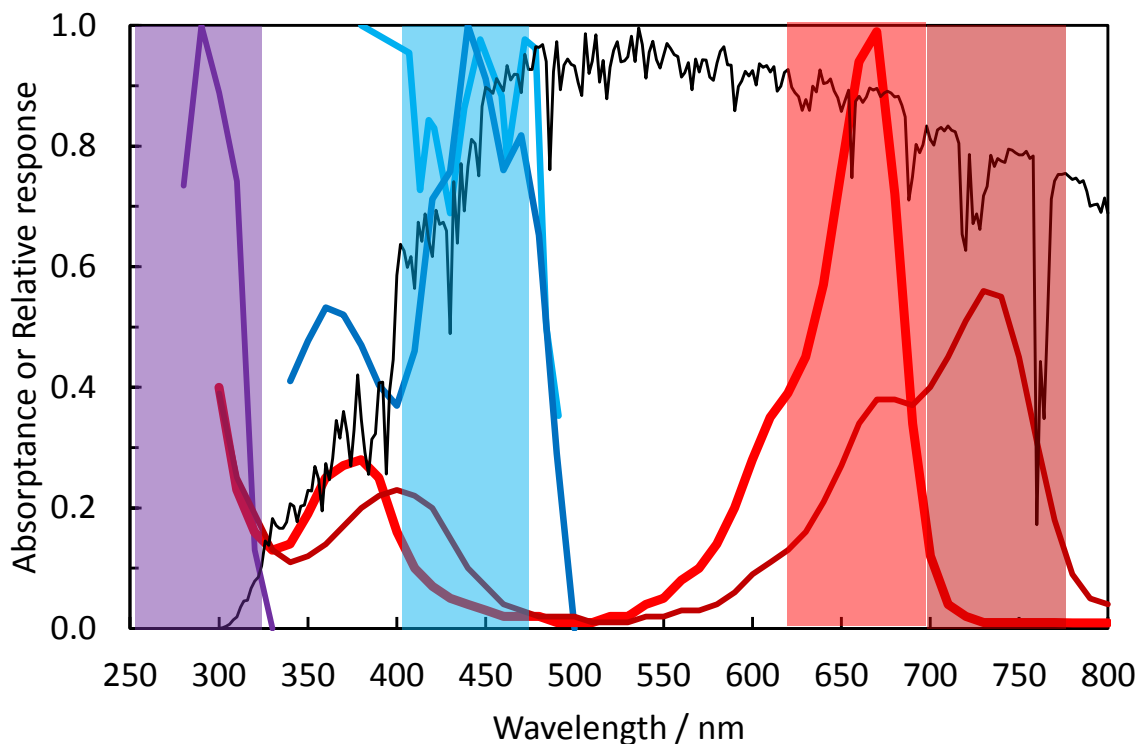


Figure 1. Plant light responses. Action spectra for UVR8 (purple line, Gardner *et al.*, 2009) cryptochrome (pale blue line, Briggs and Christie 2002) phototropin (dark blue line, Briggs and Christie 2002), and the absorption spectra of phytochrome B in its dark inactive state (dark red line) and its light activated state (red line). The black line shows the solar spectrum (expressed as relative photon irradiance) and the coloured bands indicate the regions of the spectrum with relevance to spectral manipulation for crops.

Even if optimal conditions for plants can be created, crops must be monitored to ensure pests and diseases are kept under control to prevent damage and loss of sales. It is important to understand how spectral manipulation of plants impacts both beneficial and pest insect species so IPM strategies and pollination can be maintained. As is the case for plants, light influences many aspects of insect biology (for example, circadian rhythms are entrained by light) and behaviour (e.g., light controls take-off and landing choices as well as migration direction). The light environment also has secondary impacts on insects. The emission of volatile compounds that can attract and deter insects is controlled by plant light responses. Light responses also alter the nutritional qualities of plants, and this can influence insect reproductive rates. A better understanding of how different light treatments influence insect fecundity could potentially allow the development of light treatments to reduce pest pressure or even light treatments that improve IPM strategies.

The monitoring of insect populations forms an important part of pest management strategies and sticky traps are a cost-effective management tool. The success of sticky traps, however, requires insects to be attracted towards the trap and trap colour is an important factor affecting the species that are trapped. If traps are not sufficiently attractive to insects, they will not provide a good indication of pest numbers and may even fail to trap important pest species. Trap effectiveness has previously been shown to be associated with the brightness and colour of the trap. In particular, many insects are strongly attracted to green light. The addition of green LEDs to yellow sticky traps can increase the effectiveness of traps to certain insect species (Nakamoto & Kuba 2004). No green light is present under red:blue LED light mixtures and yellow sticky traps do not appear yellow to human vision and presumably also appear different to insects. It is, therefore, expected that red:blue light mixtures will reduce the effectiveness of yellow traps. If this is the case alternative strategies may be required for the effective monitoring of pests under red:blue light environments.

Report overview

The experiments reported here are arranged in 3 work packages.

Work package 1 - General agronomy under LED lighting

This work package will examine the general agronomic practices required for plant production under LED lighting. One of the major benefits of LED lighting is their low energy consumption compared to conventional lighting systems. Their robust nature and ability to rapidly turn on and off also provides the possibility of further reducing energy consumption by either creating mobile light rigs that move over the crops at regular intervals or strobing the light to reduce energy consumption. Both these techniques can lower energy

consumption, but this comes at the cost of a lower daily light integral (DLI). The results from year one demonstrated that mobile and strobe lighting systems designed to reduce capital and electrical running costs have a negative impact on plant performance and quality. This was caused by both the reduced light available to plants but also by the fact that plants were less efficient at using variable intensity light than they were at using constant intensity light for photosynthesis and growth. The work reported here (year 2) will focus on furthering our understanding of the influence of light intensity on plant quality, growth rate and running costs. The growth of Petunias, Pansies and Lettuce were examined in this work package.

Work package 2 - Influence of light quality on crops

The experiments in work package 2 will examine the responses of plants to different light spectra with the aim of improving our understanding of the diversity of plant responses to light and to help commercial implementation of LED technologies. WP2 is divided into subsections examining different aspects of light quality on plant morphology. This report contains results from four subsections of WP2:

WP 2.1b - Influence of red : blue ratio on plant growth.

WP 2.1c - Influence of red : far-red ratio on plant growth.

WP 2.1d - Red : blue : far-red light combinations.

WP 2.3 - Improving cutting propagation.

Several species were examined (Petunia, Pansy, Lettuce, Santolina, Clematis, Iberis). Where appropriate, plants of the same species were grown simultaneously in multiple work packages. The results will be reported in groups based on work packages.

Work package 3 - Light quality and its influence on pests

During the first year we examined the use of different colours of sticky trap on pest trapping efficiency. In this report we examine the influence of light quality on aphid and spider mite performance on Lettuce and Cucumber plants grown under different light treatment. The effectiveness of biocontrol agents is also examined under different light treatments.

Material and methods

Climate in the LED4CROPS facility

The temperature in the LED4CROPS facility was maintained at 21°C throughout the experiments. The humidity and CO₂ levels were monitored but not controlled. Crops were irrigated according to crop needs (see '*Plant material and crop measurements*' section for crop-specific details). Regular irrigation was provided by the automated ebb and flood irrigation system. The irrigation solution was maintained at an EC of 2 mS/cm and a pH of 5.5-6.5 (see Table 1 for details of the nutrient solution). When required, additional water was applied to plants by hand.

Table 1. Details of irrigation feed mixture. All values are given in mg/l.

	LED4CROPS		LED container
	Desired concentration	Mean measured concentration	Measured concentration
Nitrate N	122	194	5.2*
Sulphur	59	295	174.5
Boron	0.29	0.36	0.20
Copper	0.12	0.20	0.54
Manganese	0.34	0.51	0.33
Zinc	0.34	0.60	0.98
Iron	1.17	1.70	0.65
Chloride	96	47.8	105.5
Phosphorus	28	51.2	29.2
Potassium	265	242	114
Magnesium	42	47	19.91
Calcium	148	209	78.2
Sodium	21	50	22.1
Molybdenum	0.06	NA	NA

* Total nitrogen concentration was ~100mg l⁻¹ and provided as Ureic Acid.

Leaf morphology

Total leaf area of individual plants was determined by detaching leaves and placing them on a Li-Cor Li-3100 area meter. Two types of leaf shape assessments were made. For the first and most simple assessment, the length and width of leaf blades were assessed. Petiole lengths were also measured for leaves with a defined boundary between the leaf blade and the petiole. In leaves like those found on Lettuce, where there is no distinct petiole (the leaf blade extends all the way back to the stem), only leaf length was determined. The second and more detailed assessment of leaf morphology was performed by imaging leaves using a flatbed scanner. The scanned images were subsequently

analysed by the LeafAnalyser software package, available at <http://www.plant-image-analysis.org/software/leafanalyser> (Weight *et al.*, 2008).

Leaves are rarely perfectly flat and usually exhibit some form of curvature. Leaves can be curled in two directions, displaying lateral (Figure 2A) and longitudinal curvature (Figure 2B). In lateral curvature, the sides of the leaf curl downward. In longitudinal curvature, the tip of the leaf curls downward. Leaves often curl in both directions, creating a concave surface when viewed from below. Unless otherwise stated, the measurements of leaf curvature made in these experiments assess lateral curvature. Leaf curvature was measured by determining the projected leaf width and the width of the leaf after uncurling. Curvature, or leaf curling index (CI) was then quantified as the ratio between the two measurements ($CI = \text{projected width} / \text{unrolled width}$). Flat leaves have a CI close to 1 while heavily curved leaves have values less than 0.5.

The angle at which leaves are held relative to the floor is important for the appearance of a crop but also has implications for how effectively plants can capture light. Both petiole and leaf angle are influenced by light quality. Leaf and petiole angles were determined using a protractor held against the stem of the plant. The vertical (usually in line with the stem) is assigned an angle of 0° (see Figure 2C). A leaf held parallel to the floor would have an angle close to 90° while a leaf hanging downward will have an angle greater than 90° . For leaves exhibiting longitudinal curvature, determining leaf angle can be challenging. In these cases, petiole angle is often a more robust measurement.

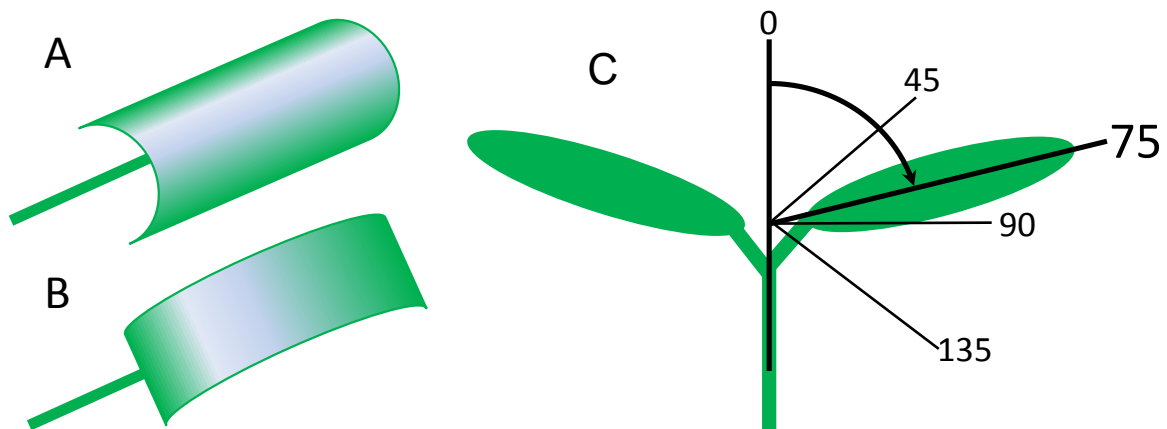


Figure 2. Diagrammatic representation of the two directions of leaf curling. **A)** Lateral curvature. **B)** Longitudinal curvature. **C)** Diagram showing how leaf lamina angle was measured.

Petunia flower development

Flowers of different developmental stages are shown in Figure 3 and the size ranges used to determine flower developmental stage are shown in Table 2. Ten flowers stage 1 from plants grown under each light treatment were marked with tags. The flowers were checked every day for 14 days and their size and developmental stage recorded. Flower and sepal size were determined as shown in Figure 4.



Figure 3. **A)** Petunia flowers at different stages of development. Developmental stage of each flower bud is given below the flowers. **B)** The same flowers as shown in A) but with the sepals removed to show the bud size. All dimensions given in cm.

Table 2. Petunia flower but size ranges and developmental states used to delineate the different stages of development.

Size range / cm	Stage
0 - 0.5	1
0.5 - 1	2
1 - 2	3
2 - 3	4
3 - 4	5
4 - 6	6
Open	7
Senesced or Senescing	8

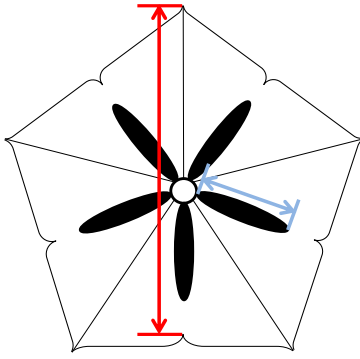


Figure 4. Diagrammatic representation of a Petunia flower viewed from the back. Red arrow shows how the flower diameter was measured and the blue lines show how the sepal length was measured.

Energy consumption and energy use efficiency

Energy consumption was calculated only for the LED energy consumption and not for the heating and cooling requirements in the LED4CROPS facility. Daily energy consumption per unit area (E_D in units of kWh m⁻²) was determined as:

$$E_D = (W_T \times h) / A \quad (1)$$

Where W_T is the total wattage of LEDs per tier, h is operational hours per day (16h in most cases) and A is the area of bench illuminated.

Crop energy consumption (E_C) was determined as:

$$E_C = E_D \times d \quad (2)$$

Where d is the number of days required to produce the crop.

For crops where plant spacing was changed during production, as was the case for bedding plants following transplantation of plug plants, energy consumption was determined per plant (E_P) as:

$$E_P = [E_{D1} \times A_{P1} \times d_1] + [E_{D2} \times A_{P2} \times d_2] \quad (3)$$

Where A_{P_x} is the area taken by each plant and the subscript number represents the different periods of production, d_x is the duration of each production period in days.

Crop energy use efficiency (U) was either determined as energy use efficiency per area (U_A) or as energy use efficiency per plant (U_P).

$$U_A = E_C / Y_M \quad (4)$$

$$U_P = E_P / Y_P \quad (5)$$

Where Y_M is the crop yield per m^{-2} and Y_p is the crop yield per plant. Unless otherwise stated yields were determined as total fresh mass of the shoot.

Light treatments

Across all experiments, photoperiod was maintained at 16 hours unless otherwise stated. Images of the different LED facilities are provided in Figure 5. Figure 6 shows the spectra of the LED lights used in the different experiments. The tables in this section indicate target light values during experimental setup. Some variation in the actual light intensities occurred during and between experiments based on location within the facility and location on the bench. All results are reported with the actual values recorded during the experiments.

WP 1.2 light treatments

All light treatments for WP1.2 were performed in the LED4CROPS facility using Philips GreenPower production DR/B modules. This experiment contained four light treatments (Table 3), each with a different electrical input and different daily light integral (DLI). The four light treatments had constant light provided at different intensities ranging from 99 to $386 \mu\text{mol m}^{-2} \text{s}^{-1}$ (see Table 2) and DLIs between 5.7 and $22 \text{ mol m}^{-2} \text{d}^{-1}$.

Table 3. Details of six light treatments trialled in Work Package 1.2.

Treatment	Low	Standard	Medium	High
Light rack	6A	5C	6B	6C
Mean PAR photon irradiance / $\mu\text{mol m}^{-2} \text{s}^{-1}$	100	200	280	360
% blue	11	11	11	11
Number of LED modules	6 X 1.5 modules	10 X 1.5 modules	16 X 1.5 modules	20 X 1.5 modules
Electrical input / W m^{-2}	80	133	213.3	266
Light field	continuous	continuous	continuous	continuous
DLI / mol m^{-2}	5.7	11.4	15.8	22.2

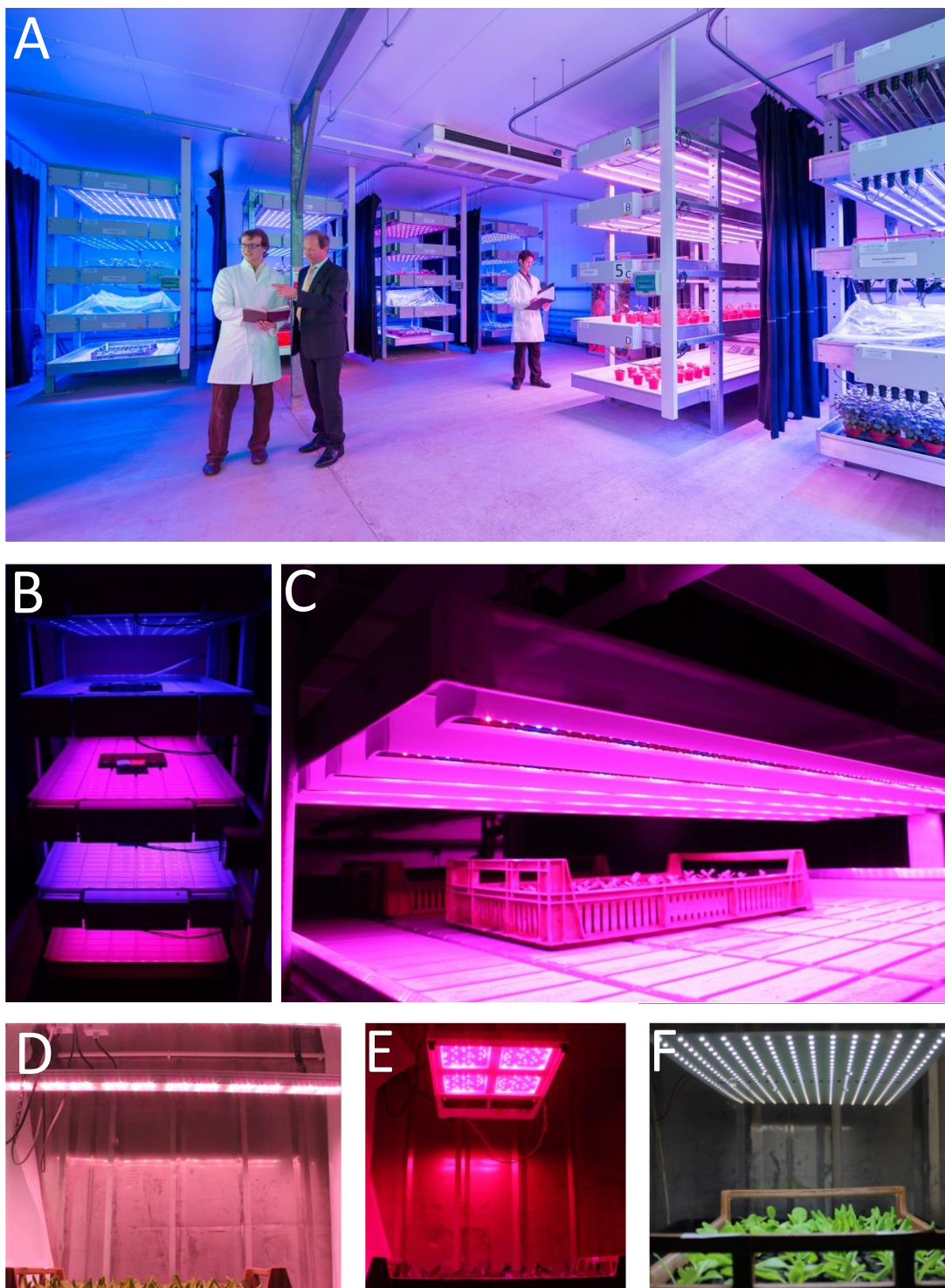


Figure 5. Images of LED facilities used in the experiments. **A-C)** Images from the LED4CROPS facility, **A)** general view of the LED4CROPS facility, **B)** a research rack with each shelf having a different red:blue treatment, **C)** the mobile light rack used in WP 1.2. **D-F)** Images from the LED container facility, **D)** a Valoya AP673 LED, **E)** a Heliospectra lamp with a red blue light treatment, **F)** a Solidlite LED lamp.

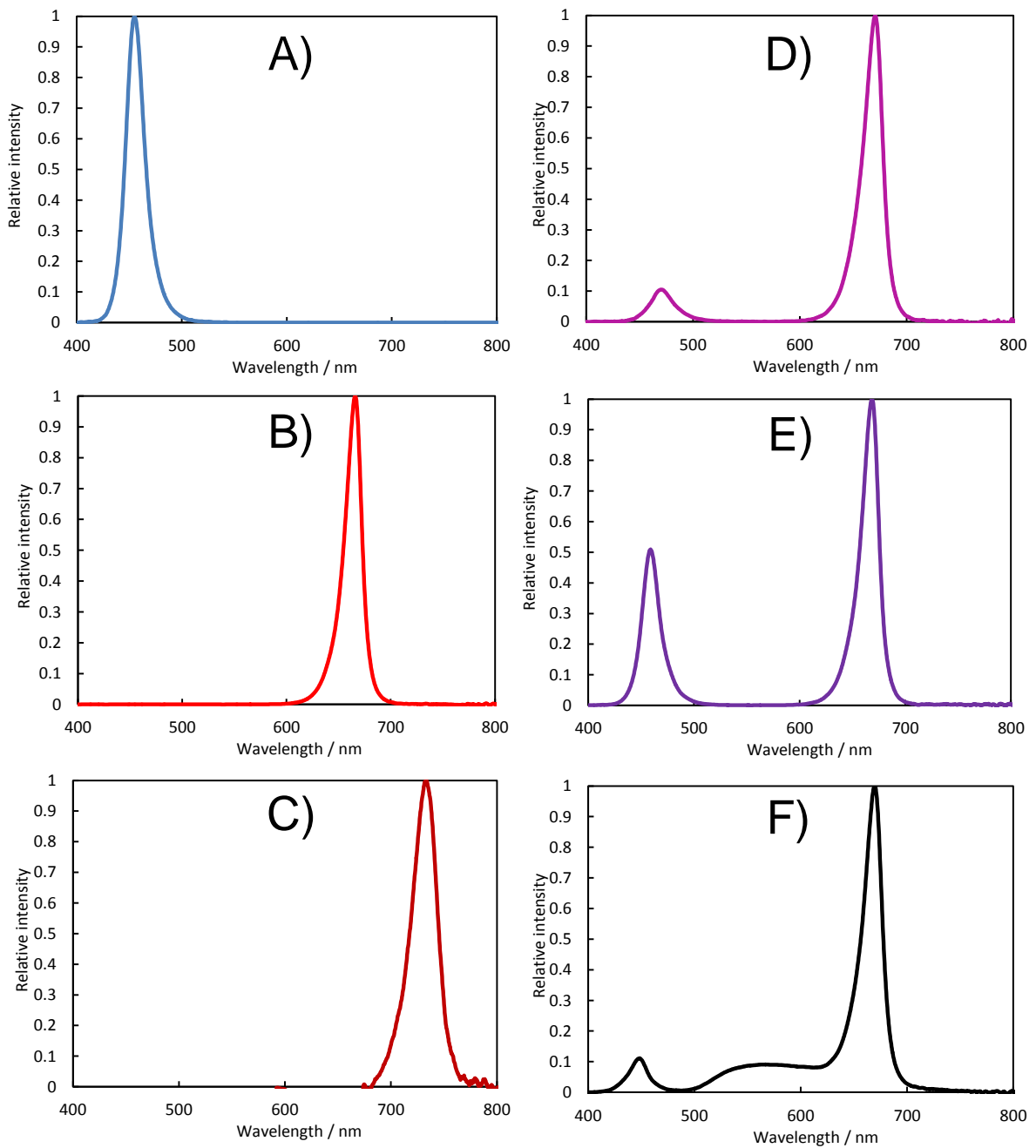


Figure 6. The spectra of the LED lights used in the LED4CROPS facility experiments.

- A) Blue Philips Research module.
- B) Red Philips Research module.
- C) Far-red Philips Research module.
- D) Red:blue Philips production model.
- E) HiBlue Philips production module.
- F) Red:white Philips production module.

WP 2.1b light treatments – Red-blue ratio

All the WP 2.1b light treatments were performed in the LED4CROPS facility using Philips GreenPower Research LED modules. The aim of these experiments was to assess the impact of different red:blue light ratios on plant development and morphology. Two colours of light were examined: red (666nm) and blue (460nm, Figure 6). Five light treatments were set up, ranging from 100% blue to 100% red (Table 4). The 100% red (referred to as 0% B) treatment was not included in earlier experiments. The intensity of the treatments was set to be close to $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. For the 100% blue light treatment, however, the maximum light intensity achieved was $145 \mu\text{mol m}^{-2} \text{s}^{-1}$. During the year one experiments, these light treatments were all located on research rack 1 (R1) on different shelves (R1A-R1D). This, however, resulted in a temperature gradient between the treatments with the bottom shelf in particular (location R1D) having a lower temperature than the other treatments. To remove this issue for the year 2 experiments the light treatments were re-organised in the facility so they were located on shelves B or C (the two middle shelves) on different research racks (R1C-R4C) where temperatures were found to be similar. For data analysis, data from an additional light treatment (the 0 far-red light treatment from WP 2.1C; see next section) were included in the analysis where appropriate.

Table 4. The specifications of the light treatments used in Work Package 2.1b.

Light treatment	100 % B	66 % B	33% B	15% B	11%B	0% B
Location	R1A or R1C	R2C or R1C	R4C or R1B	R3C or R1D	12B*	R3B
PAR / $\mu\text{mol m}^{-2} \text{s}^{-1}$	145	200	200	200	200	200
DLI / mol m^{-2}	8.4	11.5	11.5	11.5	11.5	11.5
Blue photon irradiance	145	132	66	30	22	0
Red photon irradiance	0	68	134	170	178	200
% blue	100	66	33	15	11	0

*This is the same light treatment as the lowest far-red light treatment used in work package 2.1c and is not included in the analysis of all crops.

WP 2.1c light treatments – Red:far-red ratio

All the light treatments in WP 2.1c were performed in the LED4CROPS facility using Philips GreenPower DR:B production LED modules (11% blue) with Philips Green Power far-red research modules. The aim of these experiments was to assess the impact of different amounts of far-red light in a background of red and blue light on plant development and morphology. Three colours of light were examined: red (666nm), blue (460nm), and far-red (735nm). Four light treatments were each set up, ranging from 0 to 48 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of far-red light (Table 5). The red blue light intensities were kept constant across all light treatments (200 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Initially, these light treatments were all located on light rack 1 (PR12) on different shelves (PR12A-PR12D). This, however, resulted in a temperature gradient between the treatments, with the bottom shelf in particular (location PR12D) having a lower temperature than the other treatments. To remove this issue the light treatments were re-organised in the facility so they were located on shelves B or C (the two middle shelves) of two adjacent light racks (PR10 & PR11), where temperatures were found to be similar.

Table 5. The specification of the four light treatments examined in Work Package 2.1c.

Light treatment	12B	12C	10B	10C
Measured parameters				
PAR / $\mu\text{mol m}^{-2} \text{s}^{-1}$	200	200	200	200
DLI / mol m^{-2}	11.5	11.5	11.5	11.5
Far-red / $\mu\text{mol m}^{-2} \text{s}^{-1}$	1.51	19.21	42.69	15.68
% blue	11	11	11	11
Red:far-red ratio	117	8.8	4.2	10.2

WP 2.1d light treatments – High blue and far-red

All the WP 2.1d light treatments were performed in the LED4CROPS facility using Philips GreenPower Research LED modules. The aim of these experiments was to assess the combined impact of high blue light percentages and far-red light. Three colours of light were examined: red (666nm, Figure 6), blue (460nm) and far-red (735nm). Eight light treatments were set up, four with 30% blue light plus different amounts of far-red (Table 6) and four with 60% blue plus different amounts of far-red. The intensity of the treatments was set to be close to 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Table 6. The specification of the eight light treatments examined in Work Package 2.1d.

Light treatment Location	B30 FR0	B30 FR11	B30 FR20	B30 FR35
PAR / $\mu\text{mol m}^{-2} \text{s}^{-1}$	197.2	202.9	203.3	198.5
DLI / mol m^{-2}	11.4	11.7	11.7	11.4
Blue photon irradiance / $\mu\text{mol m}^{-2} \text{s}^{-1}$	62.6	57.1	59.1	60.3
Red photon irradiance / $\mu\text{mol m}^{-2} \text{s}^{-1}$	133.8	145.0	143.4	137.4
Far-red photon irradiance / $\mu\text{mol m}^{-2} \text{s}^{-1}$	1.2	11.3	20.3	35.2
% blue	31.8	28.2	29.1	30.4
Red:far-red ratio	111.4	12.9	7.1	3.9
Light treatment Location	B60 FR0	B60 FR11	B60 FR18	B60 FR33
PAR / $\mu\text{mol m}^{-2} \text{s}^{-1}$	203.6	204.2	190.4	197.8
DLI / mol m^{-2}	11.7	11.8	11.0	11.4
Blue photon irradiance / $\mu\text{mol m}^{-2} \text{s}^{-1}$	126.8	117.2	104.2	113.9
Red photon irradiance / $\mu\text{mol m}^{-2} \text{s}^{-1}$	75.1	85.4	84.8	83.6
Far-red photon irradiance / $\mu\text{mol m}^{-2} \text{s}^{-1}$	0.5	11.0	18.5	32.5
% blue	62.3	57.4	54.7	57.6
Red:far-red ratio	137.5	7.8	4.6	2.6

WP 2.3 Improving HNS propagation

Pre excision lighting (at Kernock Park Plants)

The Santolina cuttings were collected from mother stock plants grown through the winter under three different light treatments **1) Unlit** - control treatment exposed to only natural light, **2) Supplemental** - natural light plus LED light provided at $51\mu\text{mol m}^{-2} \text{s}^{-1}$ (Philips GreenPower top lights 6% blue: 94% red) for 12 hours per day **3) Day length extension** - natural light plus LED photoperiodic lighting providing 12 hour days with (Philips GreenPower flowering lamps DR/W). Iberis cuttings were collected from plants grown under the Unlit and Supplemental treatments.

Post excision lighting (Santolina)

All the post excision light treatments were performed in the LED4CROPS facility using Philips GreenPower Research LED modules (red, blue and far-red modules) or the Philips GreenPower production LED modules (11% blue, 30% blue or red-white modules). Nine light treatments were set up to examine red:blue, red:far-red and intensity effects on cutting survival and rooting. Once the experiments were underway and humidity and condensation levels had saturated inside the plastic tents, used to keep the environment humid, light measurements were made. Details of the nine treatments are shown in table 7. **Table 7.** Details of the light treatments used in WP 2.3 for the Santolina cuttings. Measurements were made below the plastic tent, which reduced light intensity by approximately 30%.

Treatment number	1	2	3	4	5	6	7	8	9
Treatment Name	100 %R	61 % B	33%B	15%B	15%B +FR	White	Low	Med	High
Location	R2B	R1B	PR10D	R3B	R4B	P7C	P6A	P6C	PR11D
PAR / $\mu\text{mol m}^{-2} \text{s}^{-1}$	82.5	81.6	93.0	79.9	81.6	86.3	36.5	65.3	84.0
DLI / mol m^{-2}	4.75	4.70	5.36	4.60	4.70	4.97	2.10	3.76	4.84
Blue / $\mu\text{mol m}^{-2} \text{s}^{-1}$	0.09	49.45	30.95	7.18	7.07	7.71	3.84	7.51	8.56
Green / $\mu\text{mol m}^{-2} \text{s}^{-1}$	0.16	0.68	0.52	0.19	0.20	15.30	0.32	0.57	0.64
Red / $\mu\text{mol m}^{-2} \text{s}^{-1}$	82.25	31.48	61.50	72.47	74.32	63.22	32.36	57.20	74.81
Far red / $\mu\text{mol m}^{-2} \text{s}^{-1}$	0.85	0.60	0.83	0.78	14.57	1.89	0.75	0.87	1.12
Blue %	0	61	33	9	9	9	11	12	10

Red:Far-red ratio	96	52	74	93	5	33	43	66	67
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Post excision lighting (Clematis and Iberis)

For the Clematis and Iberis cuttings the locations of the light treatments were revised and reorganised. Nine light treatments were set up to examine red:blue, red:far-red and intensity effects on cutting survival and rooting. Once the experiments were underway and humidity and condensation levels had saturated inside the plastic tent, light measurements were made. Details of the nine treatments are shown in Table 8.

Table 8. Details of the light treatments used in WP 2.3. Measurements were made below the plastic tent, which reduced light intensity by approximately 30%.

Treatment number	1	2	3	4	5	6	7	8	9
Treatment Name	100% R	61% B	33%B	15%B	15%B +FR-8	15%B +FR-8	White	Low	Med
Location	R4D	R2D	R1D	R3B	R4B	R3B	P7C	P6A	P6C
PAR / $\mu\text{mol m}^{-2} \text{s}^{-1}$	78.34	71	72.65	76.82	76.08	72.96	87.36	37.80	73.49
DLI / mol m^{-2}	4.51	4.09	4.18	4.42	4.38	4.20	5.03	2.18	4.23
Blue / $\mu\text{mol m}^{-2} \text{s}^{-1}$	0.05	40.47	22.87	7.18	11.59	11.04	6.95	4.41	8.29
Green / $\mu\text{mol m}^{-2} \text{s}^{-1}$	0.10	0.34	0.33	0.19	0.17	0.16	15.99	0.31	0.57
Red / $\mu\text{mol m}^{-2} \text{s}^{-1}$	78.19	29.90	49.45	65.02	64.30	61.73	64.40	37.80	64.61
Far red / $\mu\text{mol m}^{-2} \text{s}^{-1}$	0.32	0.60	0.	0.24	8.96	15.35	1.51	0.18	0.43
Blue %	0	57	33	15	15	15	9	10.37	11
Red:Far-red ratio	242	221	226	269	7.18	4.02	42.56	214	151

Plant material and crop measurements

Lettuce

Two varieties of Lettuce seed were provided by Enza Zaden: Alega (a winter variety) and Amica (a summer variety). Seed were sown on 5 cm peat blocks and covered with vermiculite. Peat blocks were irrigated three times per day with the automated ebb and flood irrigation system to maintain peat block moisture content. Plants were grown for 3 weeks before assessment. Ten plants of each variety from each light treatment were assessed for plant fresh mass, plant dry mass, leaf number, leaf length, leaf width, and leaf shape.

Bedding plants

Petunia (*Petunia hybrida*, Mirage Blue F1) and Pansy (*Viola wittrockiana*, Dynamite Strawberry) seed were supplied by CN Seeds. Seed were sown, on the 1st October 2015, on Levington F2+sand substrate in one inch cells. Plants were transplanted when the plug plants were of sufficient size. Plants were transplanted into six-packs filled with Levington M2 substrate. Plants were irrigated as required day using the automated ebb and flood system. Plants were assessed at plug stage and once matured. Flower numbers were monitored until all treatments had achieved full flowering.

Propagation plant material

Cuttings of Santolina 'Lemon Fizz' were supplied by Kernock Park Plants. Cuttings were collected at Kernock Park Plants at the start of week 4, 2016. Plants were received on the 27th January 2016 and were planted on the 28th January 2016. Cuttings were planted in 100 cell Ellegaard trays, watered and placed under the different light treatments. Due to differences in the number of cuttings that were available from each pre-excision treatment, not all pre-excision treatments were represented in all the post excision treatments see Table 9 for details. To maintain a high humidity in each light treatment, wet capillary matting was placed on each shelf and a clear plastic tent constructed to cover the cuttings (Figure 7).

Iberis 'Absolutely Amethyst' cuttings were supplied by Kernock Park plants. Cuttings were collected week 15. Cuttings were received and planted on the 14th April 2016 at STC. Cuttings were planted in 100 cell trays filled with a substrate mixed from a 50:50 peat based substrate (Levingtons M2) : perlite mixture. Humidity was maintained by enclosing the trays in tents, wet capillary matting was not used in this trial.

Table 9. Number of Santolina cuttings from each pre-excision treatments (performed at Kernock Park Plants) placed under the different post excision light treatments in the LED4CROPS facility.

Post excision treatment		Pre excision treatment		
No.	Name	Unlit	Supplemental	Day-length extension
1	100%R	50	50	50
2	60%B	50	50	50
3	30%B	50	50	50
4	15%B	50	50	
5	15%B+FR		50	
6	White		50	
7	Low		50	
8	Med		50	
9	High	50	50	50



Figure 7. Image showing the tent constructed to maintain humidity around the cuttings.

Tip and Nodal cutting of Clematis ‘The President’ were provided by Micro Propagation services. Cutting material was collected from field grown plants week 14. Plants were received and planted on the 6th April. Cuttings were planted in 100 cell trays filled with a substrate mixed from a 50:50 peat based substrate (Levingtons M2) : perlite mixture. Humidity was maintained by enclosing the trays in tents, wet capillary matting was not used in this trial.

LED lights are energy efficient and provide opportunities to light crops in novel ways (strobe or mobile lighting) to further reduce energy consumption. However, if insufficient light is provided plant quality will be poor no matter what the spectrum of the light. Equally if too much light is provided plant quality may be excellent but the installation will be economically unviable. Achieving the correct balance between plant quality and installation/running costs is vital for sustainable production.

Each plant species is adapted to live in a specific environment and has a specific range of light requirements. Plants are generally grouped as sun, partial shade or shade plants based on the conditions that produce the best specimens. When plant light requirements are provided, they are generally given in terms of daily light integrals (mol m^{-2}) which is a measure of the total light received over a day. Under natural light conditions light intensities are highly variable ranging between 0 to $2500\mu\text{mol m}^{-2} \text{s}^{-1}$. The efficiency with which plants can use natural light is restricted by how rapidly the plant can respond to changes in light intensity and the maximum photosynthetic potential of a plant. When the light intensity increases plants must open their stomata and activate the photosynthetic processes. In bright light plants can be exposed to more light than they require, resulting in reduced light use efficiency but also potentially, damage to the plants. In LED lit environments the maximum light intensities are lower but are constant for the lit period, this may help plants use the light more efficiently. In this work package we will examine how different species respond to different intensities of LED with the aim of assessing the optimal conditions for crop production, in terms of crop quality, speed of production and electrical requirements.

The results from the year one trials examining Lettuce propagation under the mobile and strobe light treatments indicated that plants used light energy from these treatments poorly and so these treatments were excluded from this year's trials. The experiments will now focus of plant growth and development under a range of constant light intensities ranging between 100 and $360 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Results

WP 1.2 - Energy saving and daily light integral

Lettuce

The Lettuce propagation data presented in the year one report was from a single replicate set of measurements. Here we report the combined results from three replicated trials which provide a more robust assessment of the light treatment effects.

The data from the three replicate trials were similar and showed consistent trends. In all treatments the Lettuce plants were healthy, bright green and disease free. The leaves of the Amica plants were more curled than those of the Alega plants in these trials (Figure 8). For both Lettuce varieties fresh mass increased with increasing light intensity (Figure 9A), the Alega plants grew larger than the Amica plants under these experimental conditions. For the Amica variety fresh mass increased linearly with light intensity but the mass of the Alega variety increased non-linearly with light intensity. Leaf number of both varieties was observed to increase with light intensity up to $280 \mu\text{mol m}^{-2} \text{s}^{-1}$ (DLI 17 mol m^{-2}) but not to increase as the light intensity increased further (Figure 9B). Interestingly leaf length was observed to decrease as light intensity increased (Figure 9C). Leaf width was similar for the two varieties and was observed to be greatest for the two intermediate light intensities (Figure 9D). The leaves of the plants were more robust in the higher light intensity treatments and this was reflected in the measurements of leaf mass area (LMA, Figure 9E). The large differences in leaf curling between the two varieties were highlighted by the leaf curling data (CI, Figure 9F).

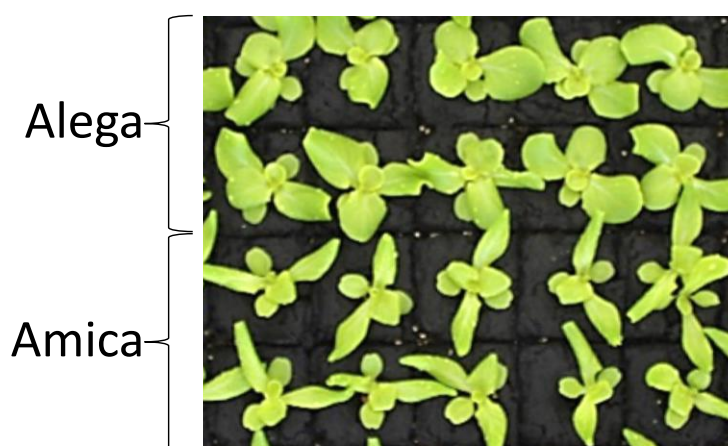


Figure 8. Photograph of the two Lettuce varieties showing the differences in leaf curling.

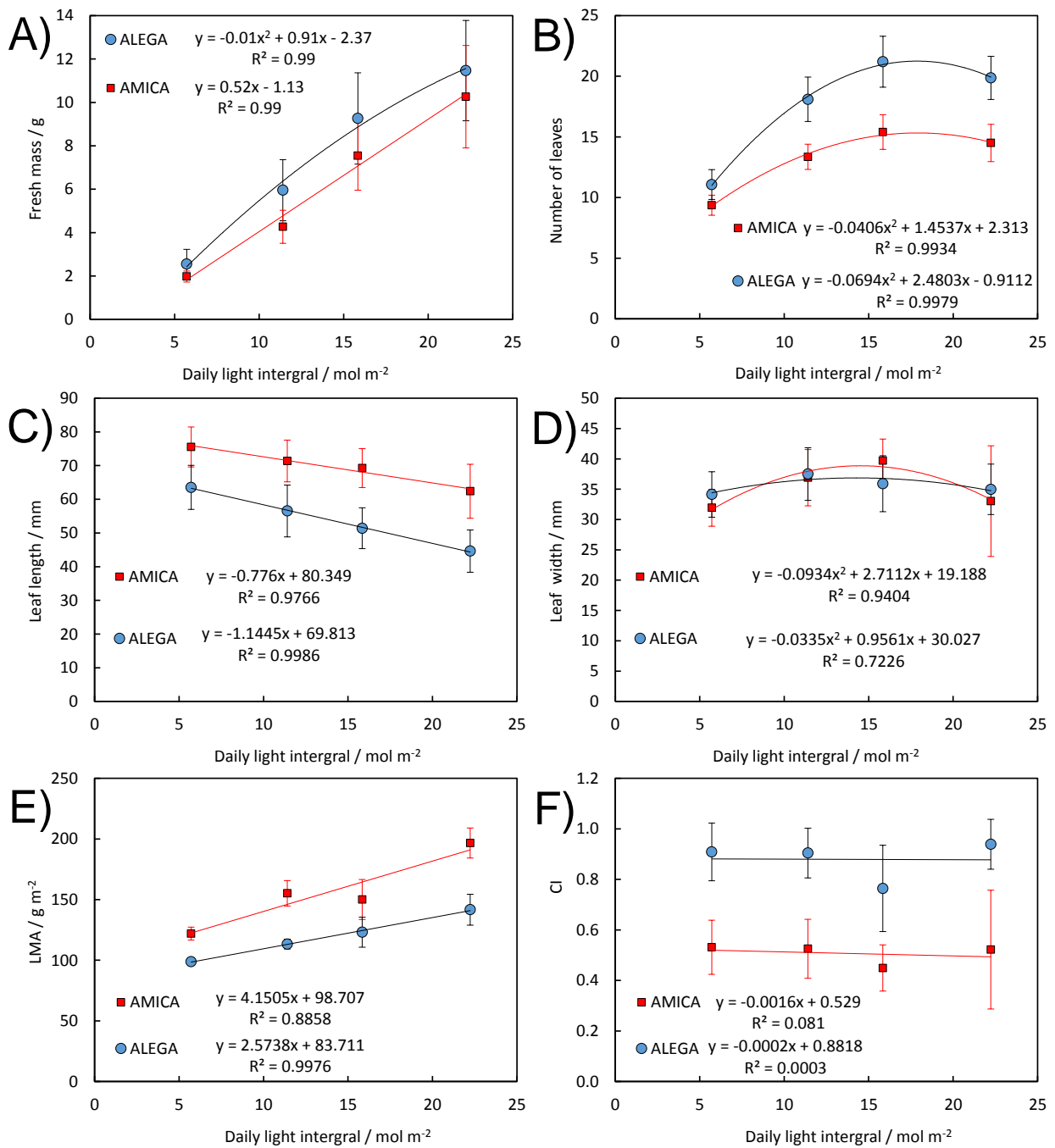


Figure 9. The influence of daily light integral (DLI) on the **A)** fresh mass, **B)** number of leaves per plant **C)** length of the fifth leaf, **D)** width of the fifth leaf, **E)** the leaf mass per area (LMA) and **F)** the leaf curling index for the two Lettuce varieties. Error bars indicate the standard deviations calculated from all the measurements of the three replicate trials.

The light intensity and/or daily light integral had clear effects on the morphology and growth rates of the Lettuce plants with growth rate and plant robustness increasing with light intensity. But how effectively do the plants use the light energy and what intensity produces the most energy efficient system. To assess this we determined both the life-time light use efficiency (LLUE) and the electrical energy use efficiency (EUE) for the two varieties grown under the four light treatments (Figure 10A and 10B). The two varieties responded differently with the Alega reaching maximum efficiency (both for LLUE and EUE) at 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (11 mol m^{-2}) and remaining similar for higher light intensities. In contrast the efficiencies of the Amica plants was found to increase almost linearly with increasing light intensity. The differences in the responses of the two Lettuce types was potentially caused by the different leaf morphologies as the curled leaves of Amica plants would intercept less light than the flatter Alega leaves. If the DLI is multiplied by the leaf curling index (CI) the LLUE and EUE curves become more aligned (Figure 10C and 10D).

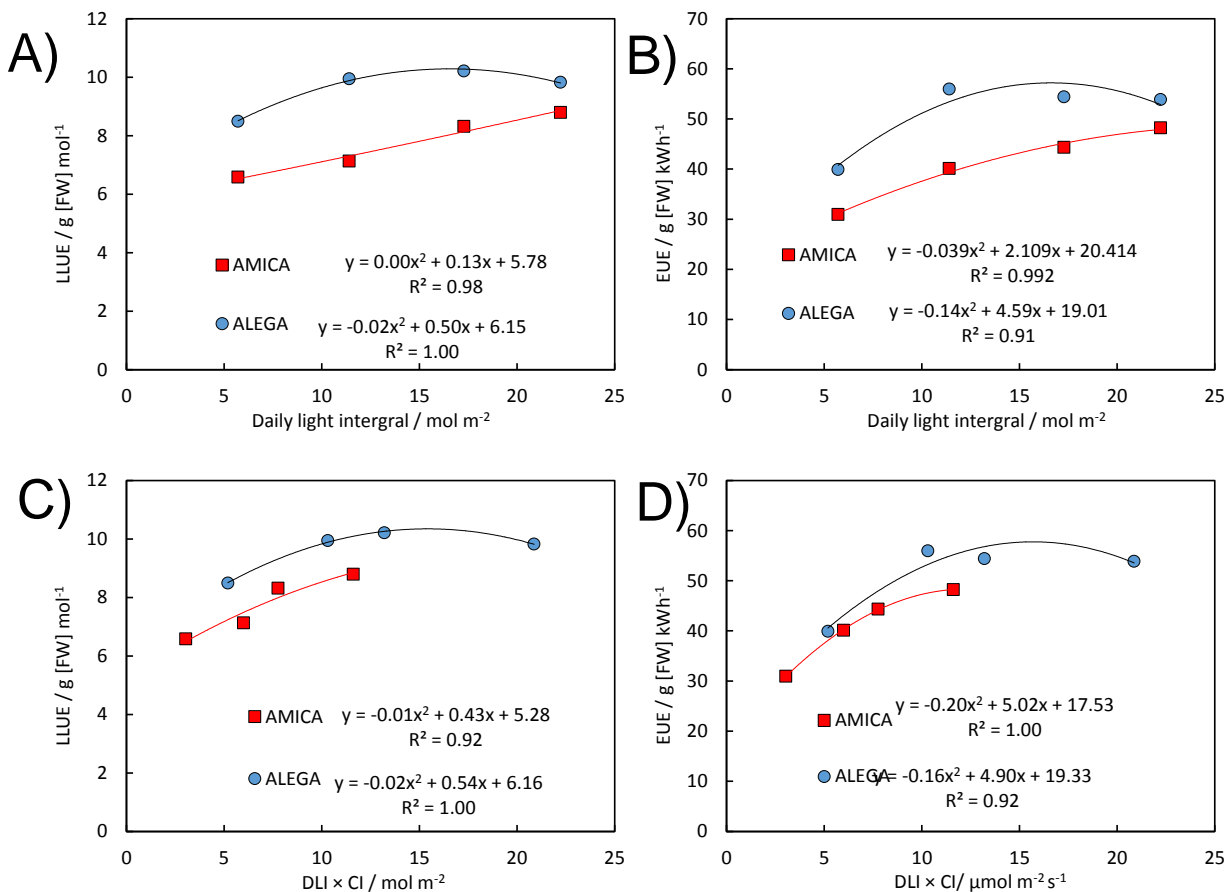


Figure 10. The influence of daily light integral (DLI) on Lettuce **A)** life-time light use efficiency (LLUE) and **B)** electrical energy use efficiency (EUE). **C)** and **D)** show the same data as presenting in A) and B) but with the DLI values scaled to account for differences in leaf curling (CI).

Petunia

Petunia plants were grown under four different light intensities (100, 200, 280 and 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Plant growth was observed to increase rapidly as the light intensity increased. After 26 days of growth the plants in the lowest light intensity (100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were small and plugs disintegrated upon inspection due to limited root development (Figure 11). At this time all the other plants had achieved sufficient size to require transplanting. The plugs from the 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment remained intact on inspection but were less robust than those from the two higher intensity treatments. Leaf size was observed to increase between 100 and 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but decrease as the intensity increased further to 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$. While the leaves of the plants in the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment were smaller these plants were more robust than those grown under less light. All treatments were transplanted to six-packs after 26 days of growth. Following transplantation plants grew rapidly and plants from the highest intensity treatments produced open flowers 9 days after transplanting, 35 days after sowing (Figure 12). After 43 days growth the plants from both the 280 and 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments were flowering rapidly but remained very compact in morphology (Figure 13). The plants from the 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment were also beginning to flower but those from the lowest light intensity were only just beginning to produce flower buds.



Figure 11. Petunia plug plants grown for 26 days (photographs taken on the 27th October 2015) under different light intensities.



Figure 12. Petunia plants grown under different light intensities for **A)** 26 days (27th October 2015) immediately after transplantation to six-packs and **B)** 35 days (5th November 2015) nine days after transplantation to six-packs.



Figure 13. Petunia plants grown under different light intensities for 43 days. Photographs taken on the 13th November 2015 two weeks after transplantation to six packs.

After 43 days the Petunia plant mass was found to increase linearly with light intensity

(Figure 14). Overall quality of the plants was also found to correlate with light intensity. Leaf mass area (LMA) was observed to increase with light intensity indicating that plant robustness increased. The plants from the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment had particularly thick feeling leaves. While mass increased with intensity internode length was observed to decrease showing plants to also be more compact in habit. In addition to the robust nature of the plants, flowers when produced, remained on short petioles and overall plant morphology remained compact even when flowering was advanced. Numbers of side shoots were similar in all treatments.

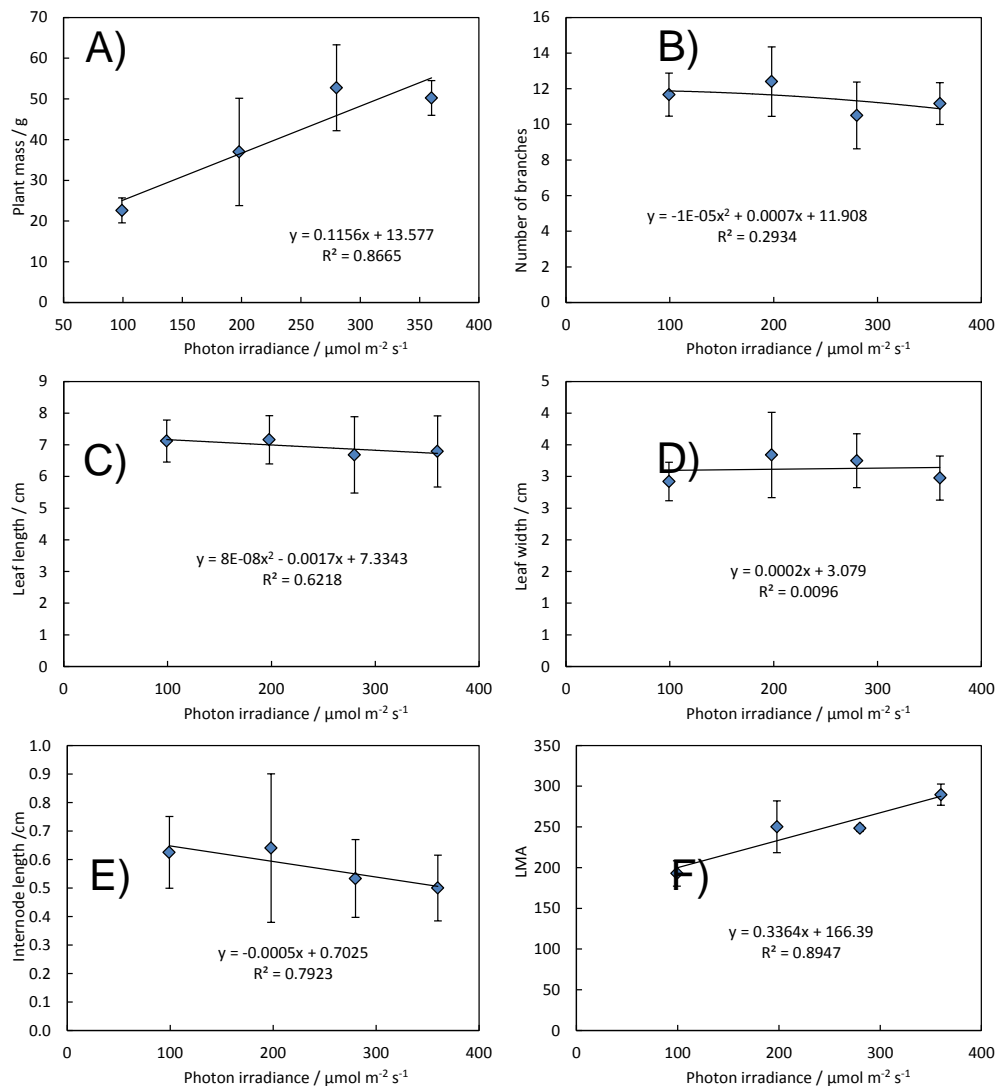


Figure 14. The influence of light intensity on Petunia plant mass and morphology after 43 days of growth. **A)** Mean plant mass. **B)** Mean number of side branches. The mean leaf length **C)** and width **D)** of the leaf below the first flower (the last vegetative leaf). **E)** Mean length of the internode below the last vegetative leaf. **F)** The mean leaf mass area (LMA) of the last vegetative leaf.

Figure 15 shows the time course of Petunia flower production in the four light intensity treatments. Total numbers of visible buds were similar in the 280 and 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light treatments though more buds were observed in the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment during the last ten days of measurement. The 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment had between a quarter and a third of the flower buds observed in the highest light intensity treatment. The number of open flowers also correlated with light intensity with the greatest numbers of flowers observed in the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment. Flower development in the 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment was observed to be between 3 and 4 days behind those in the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment while the 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ plants were 11 days behind those in the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment. Flower development in the 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment plants was over 23 days behind those in the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment and few plants had open flowers even after 58 days growth.

Total numbers of buds is only one aspect of plant development that affects flowering. The speed of transition from bud to flower and how long the flowers are open for also influences appearance. To further improve our understanding of how rapidly flowers change from buds to flowers ten flower buds 2mm in length (stage 1 flower buds) from each treatment were labelled and assessed every day over a two week period. The rate of flower development was similar in the 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments but was one day slower in the 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment. Flowers remained open for three to four days before senescing. The diameter of open flowers was also found to correlate with light intensity (Figure 16). Interestingly sepal length was observed to correlate inversely with light intensity.

The results reported in this section demonstrate the benefits of increasing light intensity on the speed of growth and flowering as well as plant quality of Petunias. Adding more light to a system, however, requires greater investment in lamps and electricity. With this in mind how efficiently does this system convert electrical energy in to saleable plant material, how much energy is consumed in the process and do shorter production times save energy? Using the data from Figure 15B we estimated the number of days it took to produce Petunia plants with 2 flowers (Figure 17A). The 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment produced no flowers and so is excluded from this analysis. The time taken to produce two flowers decreases from 58 days to 41 days (17 days or 30% quicker) as the intensity is increased from 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$. By combining the time required and the energy inputs for crop production total energy inputs were determined Figure 17B. The energy consumption was

observed to increase with light intensity and the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment consumed 41% more energy than the 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment.

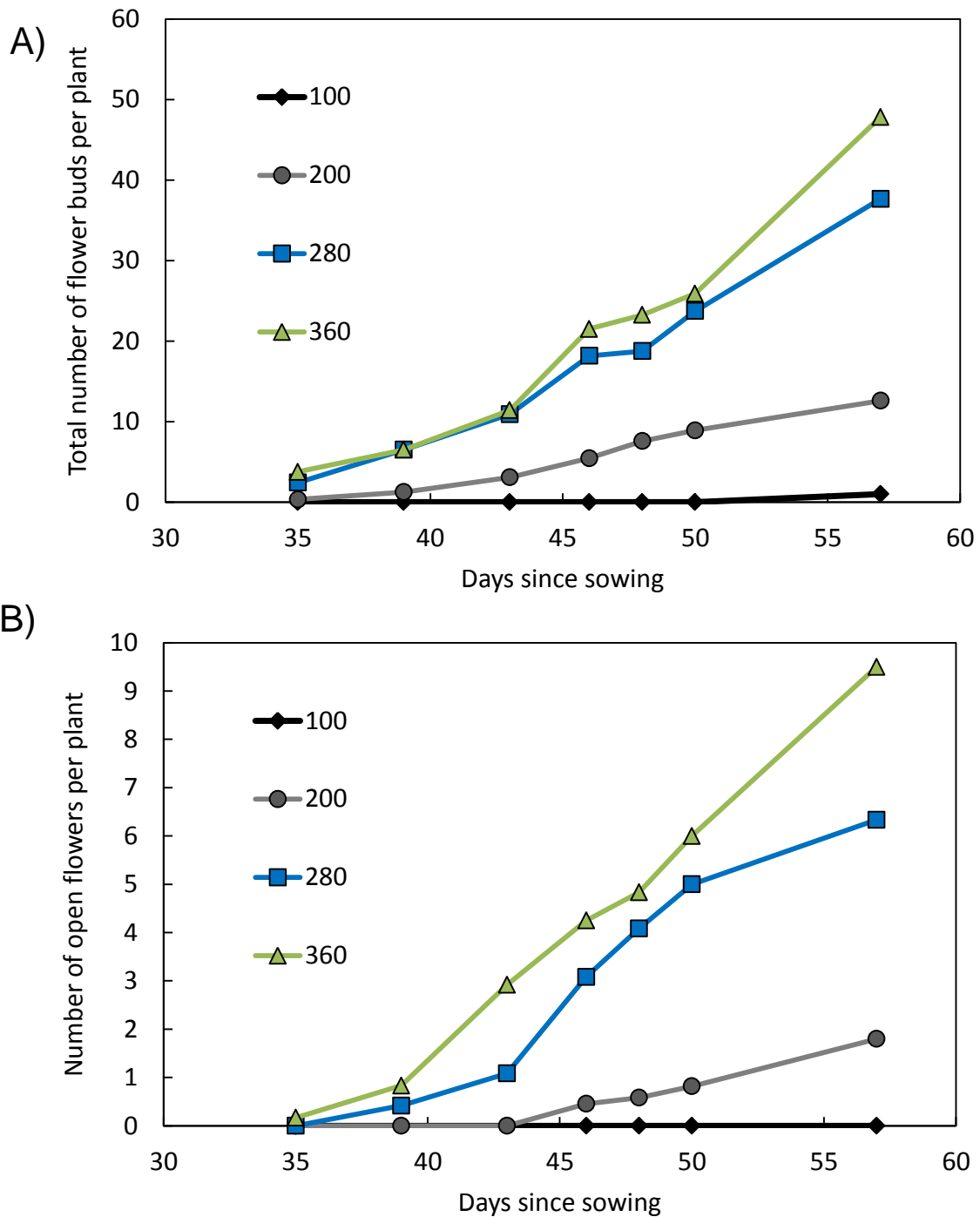


Figure 15. Influence of light intensity on Petunia flower production. **A)** Total number of visible flower buds per plant. **B)** Number of open flowers per plant.

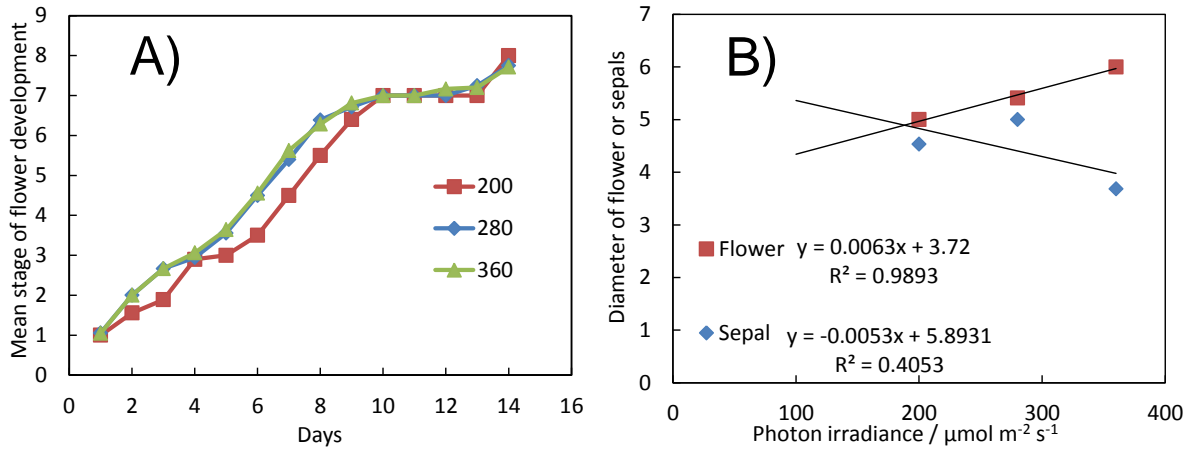


Figure 16. Effect of light intensity on **A)** the time course of flower bud development over a two week period and **B)** the size of flowers and sepals.

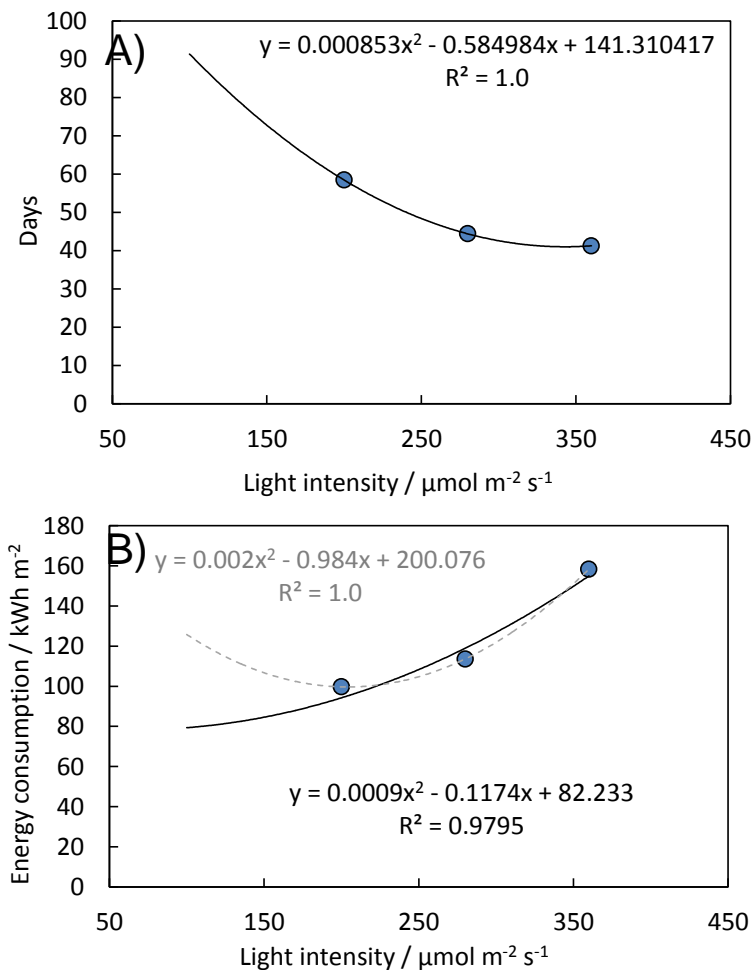


Figure 17. The influence of photon irradiance on **A)** duration required and **B)** electrical energy required to produce Petunia plants with 2 flowers. Solid line in A) represents a best fit polynomial regression. The grey dashed line in B) represents a best fit polynomial regression while the solid line shows the relationship calculated using the regression line from A).

Pansy

The Pansy plug plants from the four light intensity treatments were similar in appearance (Figure 18) but with some distinct differences. Petiole length decreased with increasing light intensity. The plants under the $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ treatment were showing slight signs of shade avoidance syndrome while the plants from the $360 \mu\text{mol m}^{-2} \text{s}^{-1}$ treatment looked perhaps too compact and bit stressed. The $360 \mu\text{mol m}^{-2} \text{s}^{-1}$ treatment resulted in the lowest numbers of usable plug plants possibly because the light intensity was too high especially soon after germination. Plants from the $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $280 \mu\text{mol m}^{-2} \text{s}^{-1}$ treatments were very similar in appearance. The Pansies were all potted up to six-packs on 10th November 2015, 41 days after sowing.



Figure 18. A) Pansy plug plants grown under four different photon irradiances. Photographs taken on the 10th November 2015 (41 days after sowing). **B)** Photographs of the Pansies from the different light treatments 3 days after (13 November 2015, 44 days after sowing) they had been potted up unto six-packs.

At the final harvest of the Pansies the plants from all treatments had grown well and had similar appearances (Figure 19). Plant mass correlated positively with light intensity (Figure 20A). Number of side branches produced per plant increased slightly with increasing light intensity (Figure 20B). Internode lengths were observed to decrease with increasing light intensity (Figure 20C) resulting in greater plant compactness. Leaf colour also varied with light intensity with leaves having a darker green colour (Figure 20D) and more robust feel at higher intensities. Leaf morphology was influenced by light treatments with petioles decreasing in length as intensity increased, especially between 100 and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments (Figure 20E). Leaf blade length was observed to be similar between 100 and 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but to increase slightly in the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment (Figure 20F).

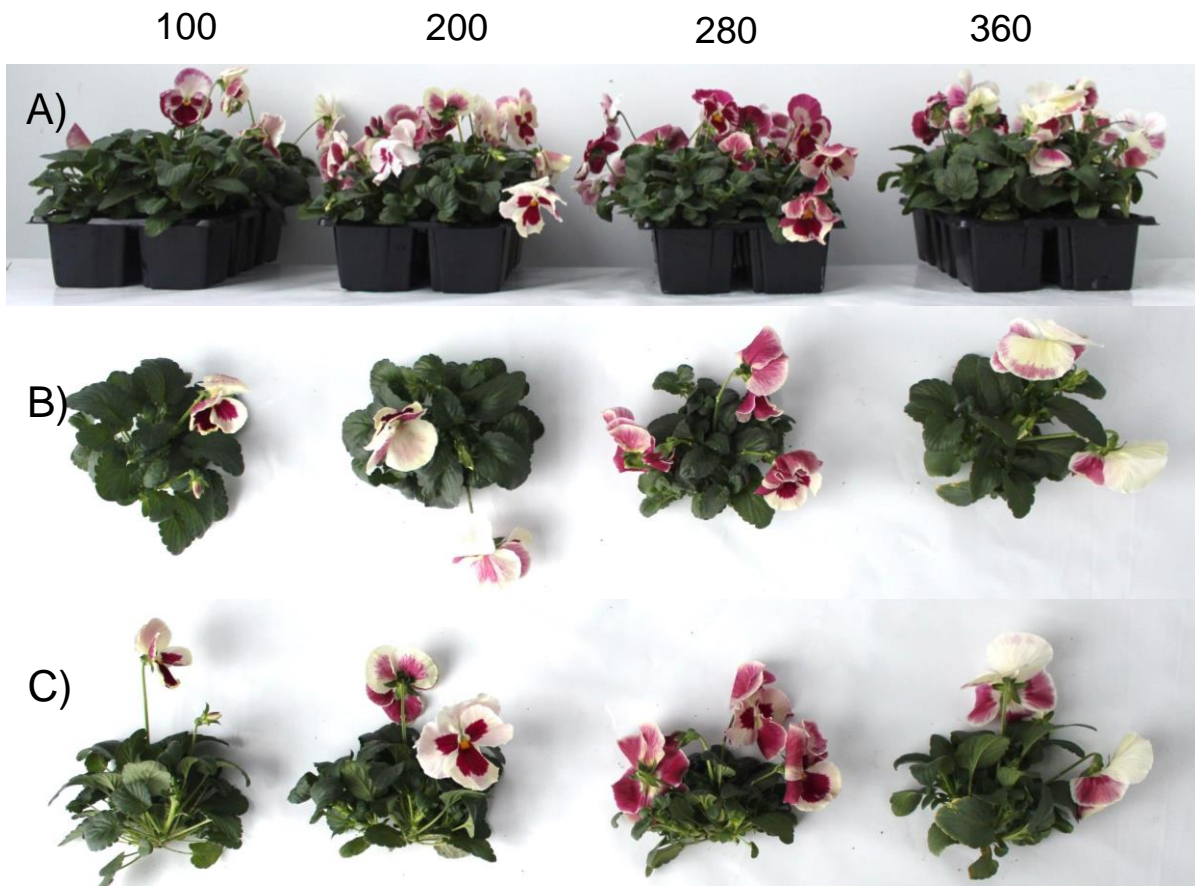


Figure 19. Pansies from the four photon irradiance treatments taken shortly after the final harvest on the 14th December 2015, 74 days after sowing. **A)** Representative six-packs of plants from the four light intensity treatments. Individual plants viewed from **B)** above **C)** from the side.

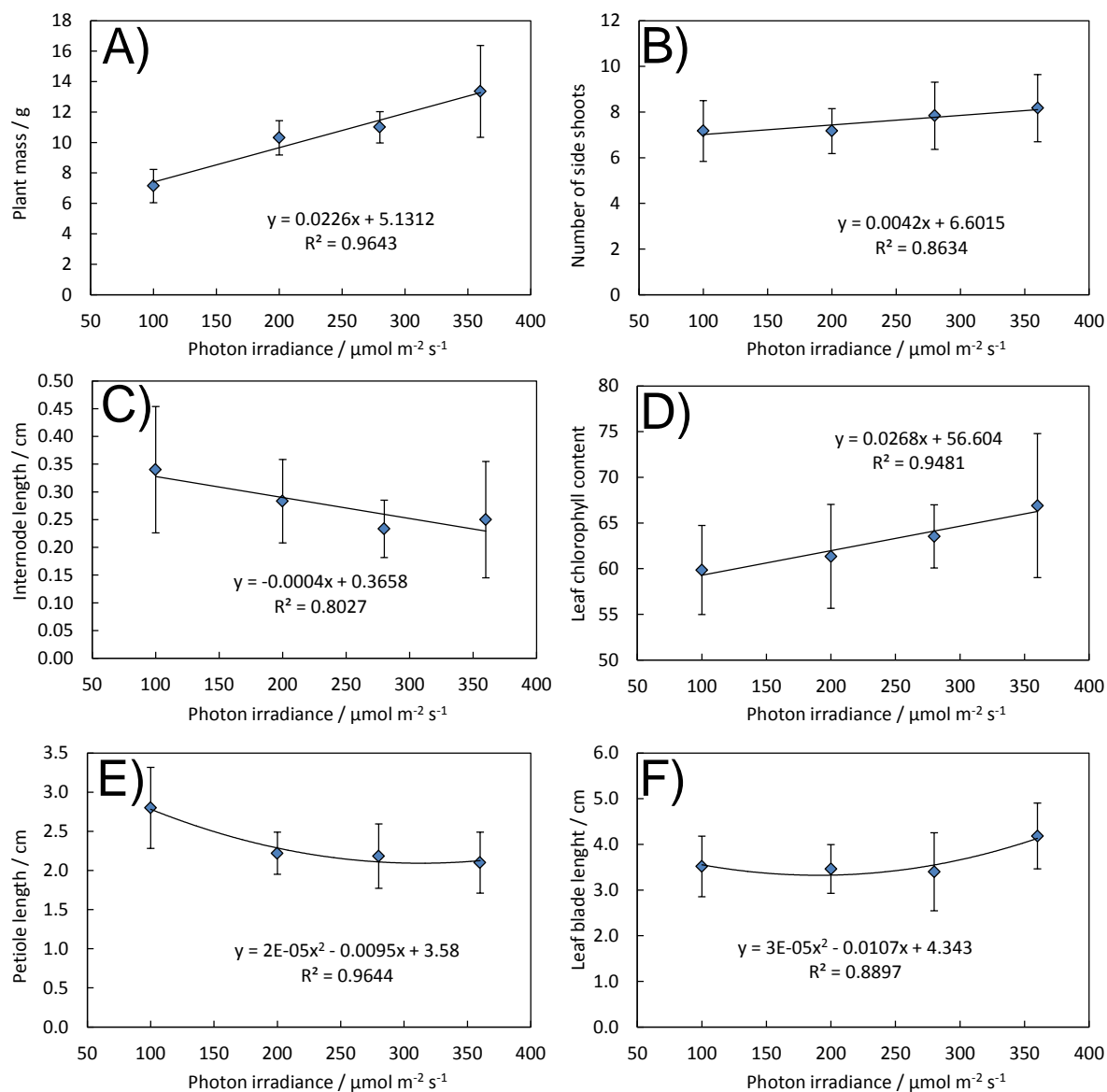


Figure 20. Influence of light intensity on the morphological parameters of Pansy plants. **A)** Mean plant mass. **B)** Mean number of side shoots per plant. **C)** The length of the two internodes found below the node holding the first open flower. **D)** Estimate of leaf chlorophyll content made using a leaf chlorophyll content meter. The mean petiole **E)** and leaf blade **F)** length of the leaf located on the node at which the first open flower was located.

Flower development in the Pansies was also influenced by light intensity. The total number of flower buds per plant was similar between the 280 and 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments, slightly lower in the 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but considerably lower for the 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment (Figure 21A). Open flowers were first observed in the 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment followed by the 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ then the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment. The 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment produced flowers last. While the 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment was third to flower, at the end of the study this treatment had produced the most flowers (Figure 21B) and flower

number correlated with light intensity. Increasing light intensity was also found to increase the diameter and mass of the Pansy flowers (Figure 22). Flower spike length was found to decrease between 100 and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but remain similar at higher intensities. Sepal length changed little between treatments.

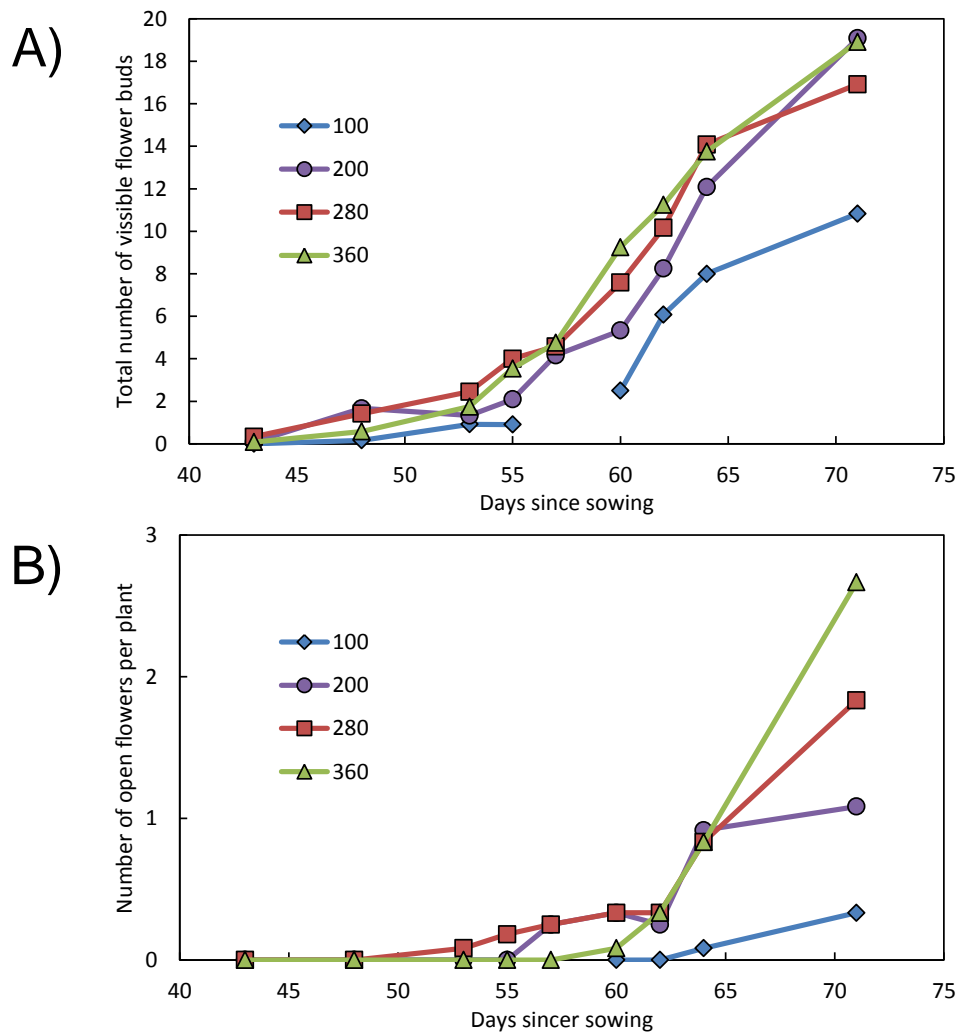


Figure 21. Time course of flower development in Pansies grown under different light photon irradiances. **A)** Mean number of flower buds visible per plants. **B)** Mean number of open flowers per plant.

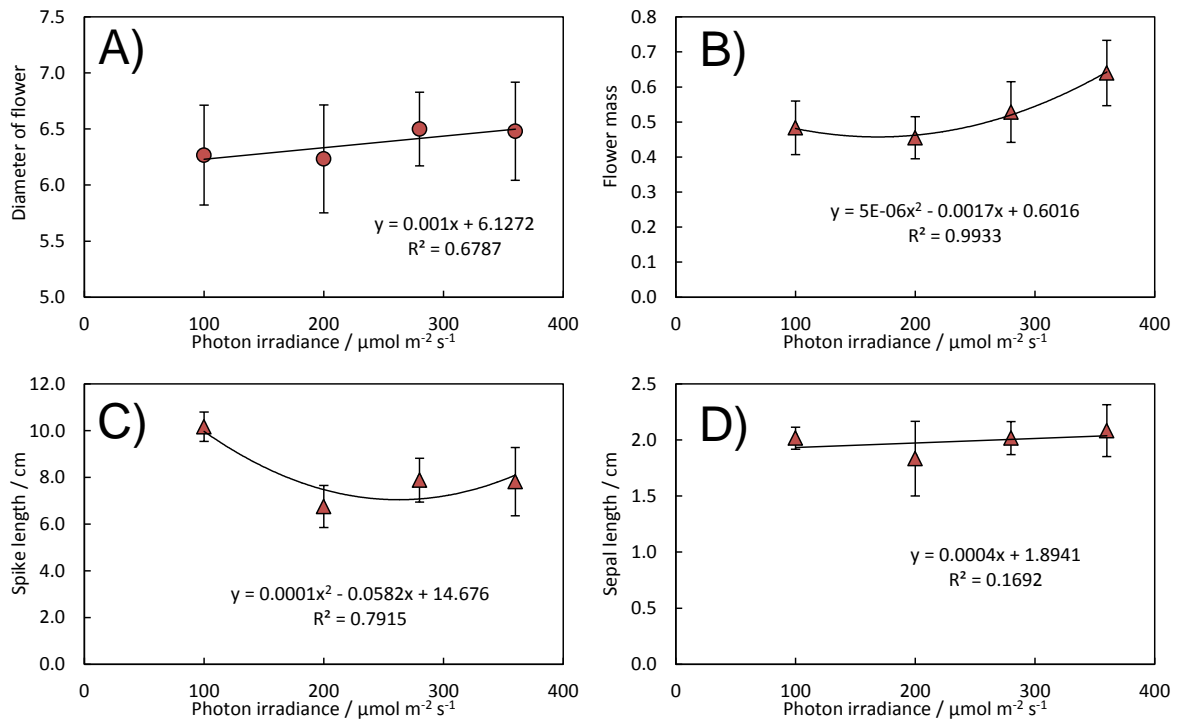


Figure 22. The influence of photon irradiance on Pansy flower size and morphology.

To assess the influence of light intensity on energy consumption required to produce flowering Pansies we used the data from Figure 21 to determine the time required to produce plants with one flower per plant (Figure 23). It was estimated that under the $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ treatment it would take 90 days to produce a Pansy with one flower. This duration dropped to 67.5 days at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. Between $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $360 \mu\text{mol m}^{-2} \text{s}^{-1}$ this period only decreased by a further 3 days. Energy consumption required to produce plants with one flower (Figure 23B) was observed to be similar between 100 and $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ but to increase as light intensity increased at intensities above $200 \mu\text{mol m}^{-2} \text{s}^{-1}$.

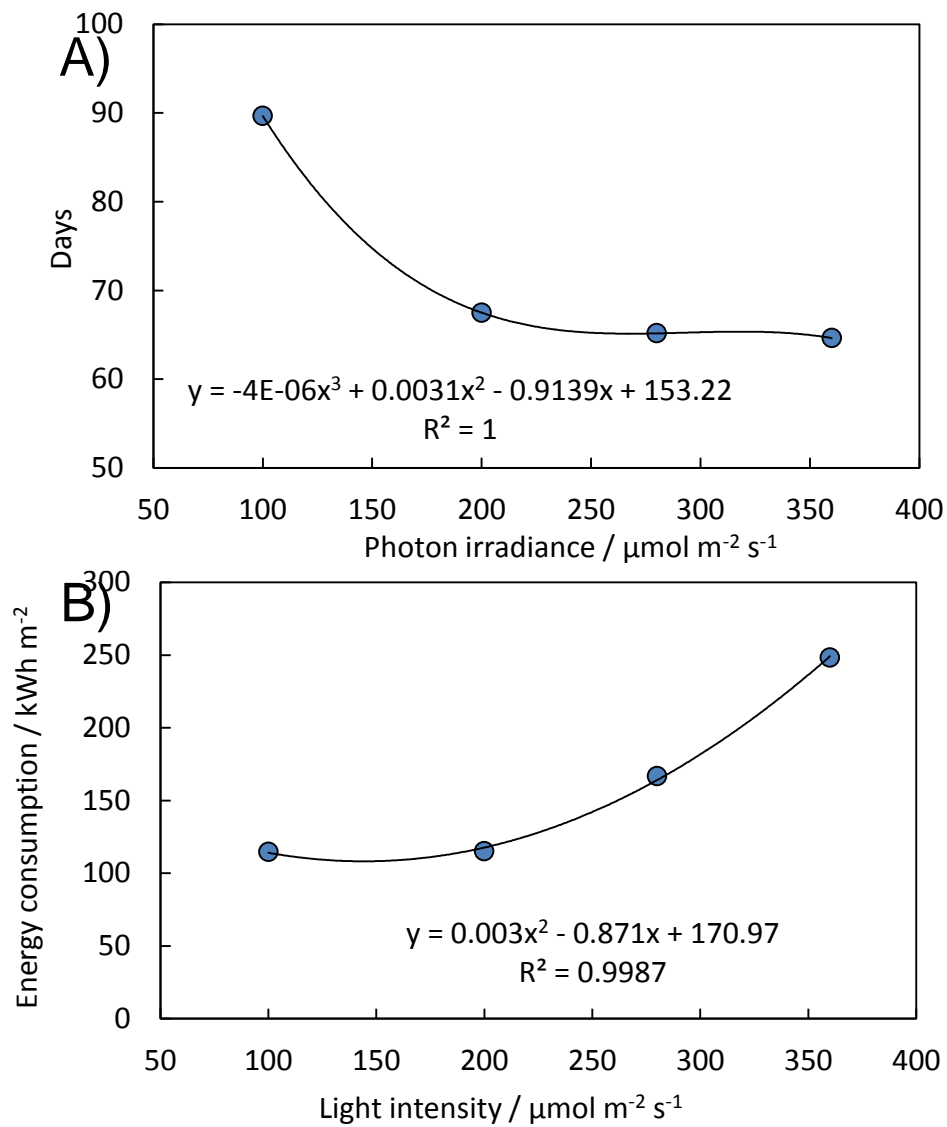


Figure 23. The influence of light intensity on **A)** duration of time required and **B)** electrical energy required to produce Pansy plants with 1 flower.

For the three species examined in these experiments plant mass was observed to increase as the total amount of light incident on the plants increased. This suggests that the additional increases in light intensity could have resulted in further increases in growth rate. For the Alega Lettuce plants the growth rate under the highest light intensity was slightly below the linear relationship observed for the other Lettuce variety, Amica, and the Pansy and Petunia plants investigated. There are two possible causes for this dip in growth rate: 1) the light intensity was approaching the point of light saturation for this variety, the intensity above which no additional photosynthesis can occur, or 2) the close plant spacing used in this propagation trial caused competition for light between the individual plants. While both possibilities are likely to contribute to some extent, in this trial it was most likely that the spacing was the major cause of the dip in growth rate and that the plants were ready for transplantation 3-4 days before the end of the three week experiments. Optimised spacing could contribute to higher growth rates and maximum plant production per unit area. Clearly there will be a need to match spacing/transplanting times to the light intensity and growth rates. If done correctly this could maximise the output of plants produced by an LED facility. Rate of plant production will have a significant impact on the economics of operating such a facility as it impacts the area that needs to be lit to produce the number of plants required. Selection of appropriate varieties will also be important to achieve maximum growth rates. This is apparent from the differences in light use efficiency and growth rates of the two Lettuce varieties which are thought to be caused by a combination of differences in leaf shape (which impacts light capture) and other physiological factors associated with differences in summer and winter Lettuce varieties (Amica is a summer variety and Alega a winter variety).

While increasing light intensity had a near linear influence on biomass production some of the other parameters that control the production period did not respond linearly. Speed of flowering in Pansy and Petunia was observed to increase markedly between 100 and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but less so as intensity increased to 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$. So in these cases the higher intensity treatments increased energy consumption which increased running costs but for less decrease in production time. From an energy perspective these results suggest that 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment provided the best balance between speed of production and energy input. However, increased energy inputs had some important impacts on plant quality. Plants from the higher intensities had improved plant habit (increased compactness with thicker more robust leaves) and greater numbers of larger flowers, both factors that could influence the crop sales. These plants would be expected to

have greater ability to resist the stresses that would be encountered following transplantation to garden/field settings and may also provide benefits to shelf life while at retailers. This highlights the trade-off between plant quality and energy inputs that underpin the economics of plant production under LED lights. These results provide the first steps in defining the influence of light intensity on plant quality and running costs. While these data can be used to develop the methods to assess the economics there will always be a need for small scale, on-site trials prior to making larger scale investments.

This data also demonstrates the potential to use light intensities to control plant habit with the aim of reducing the need for plant growth regulators during production; more light equals more compact plants. However, once the plants are removed from these light treatments any growth restriction provided by the lights will be released and any new growth will revert to match the prevailing conditions. This may mean that while no PGRs are required during the production phase a single PGR application may be beneficial prior to sale to maintain the compact morphology while plants are exposed to sub-optimal conditions during transport and sale.

Conclusions WP 1.2 - Energy saving and daily light integral

In these experiments higher light intensities were associated with faster growth, increased compactness, more rapid flowering and thicker more robust leaves. Overall quality of the petunia and lettuce plants was greatest under the highest light intensity ($360 \mu\text{mol m}^{-2} \text{s}^{-1}$). For pansy the higher light treatments resulted in most flowers but the plants were too compact. While higher light intensities can result in faster growth and earlier flowering the greatest energy efficiency was observed at a light intensity of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. This means that increasing plant quality by increasing light intensity comes at a cost, both a greater capital expenditure on lamps but also a greater running cost. These increased costs would have to be balanced versus any potential increased returns based on greater output or price per unit and any reductions in PGR requirements. Providing different light intensities at different stages of growth may enable production of the best quality plants for lower costs.

Results WP 2.1b - Influence of red / blue ratio on plant growth

Lettuce

Two varieties of Lettuce Amica and Alega were grown under different red: blue light mixtures ranging from 100% blue to 0% blue (100% red) light. The data presented are from three replicated experiments. Fresh mass of both varieties was strongly responsive to blue light percentage with the greatest biomass occurring at 15 % blue light (Figure 24A). Biomass dropped rapidly between 15 and 0% blue light, demonstrating the plant requirements for blue light. Leaf number was found to be greatest under 15 % blue light and to decrease as blue % increased and decreased (Figure 24B). Variations in leaf length in response to blue light were very different in shape to the biomass response with the shortest leaves occurring under the 60% blue light treatment and the longest under the 100% blue light treatment (Figure 24C). The leaf width data showed some of the characteristics of both the biomass and leaf length responses to blue percentage. The leaf width was reduced under 100% red light (especially in Amica) but leaf width was otherwise lowest in 60% blue light and highest in 100% blue light (Figure 24D). Leaf mass area, an estimate of how thick and robust the leaves are was greatest under the 60% blue light and equally low in the 100% red and 100% blue light. The Amica leaves were consistently thicker than those of the Alega plants (Figure 24E). Leaf chlorophyll content estimated using a chlorophyll content meter was greatest under the 60% blue light treatment and lowest under the 100% red and 100% blue light treatments (Figure 24F).

Petunia

We examined the influence of red:blue ratio on the rate of flower development in Petunia. Plants were grown under red blue light mixtures with 11, 30 and 60% blue as well as a white light containing 6% blue. Ten stage one flower buds were marked and their developmental stage determined every day over a two week period (Figure 25A). Flowers were observed to develop and open more rapidly under light treatments with higher blue light percentages (Figure 25). While the speed of development was faster in the high blue light treatments the total life span of the majority of the flowers remained at 14 days or less after labelling. This indicates that the flowers also remained open for longer under the high blue treatments compared with the lower blue treatments.

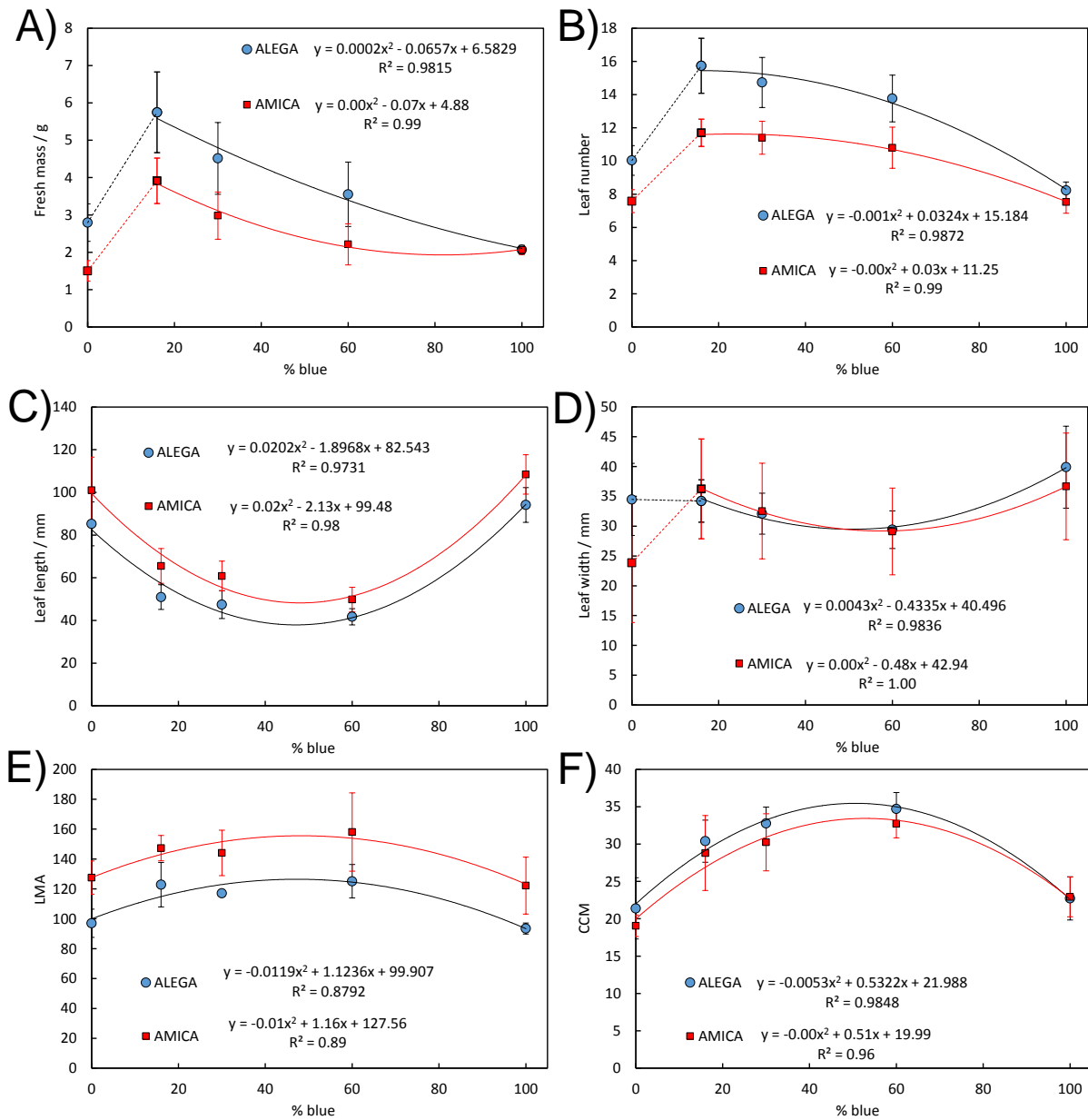


Figure 24. The influence of blue percentage of a red:blue light mixture with an intensity of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ on the growth and morphology of two Lettuce varieties (Amica and Alega). Error bars indicate standard deviation.

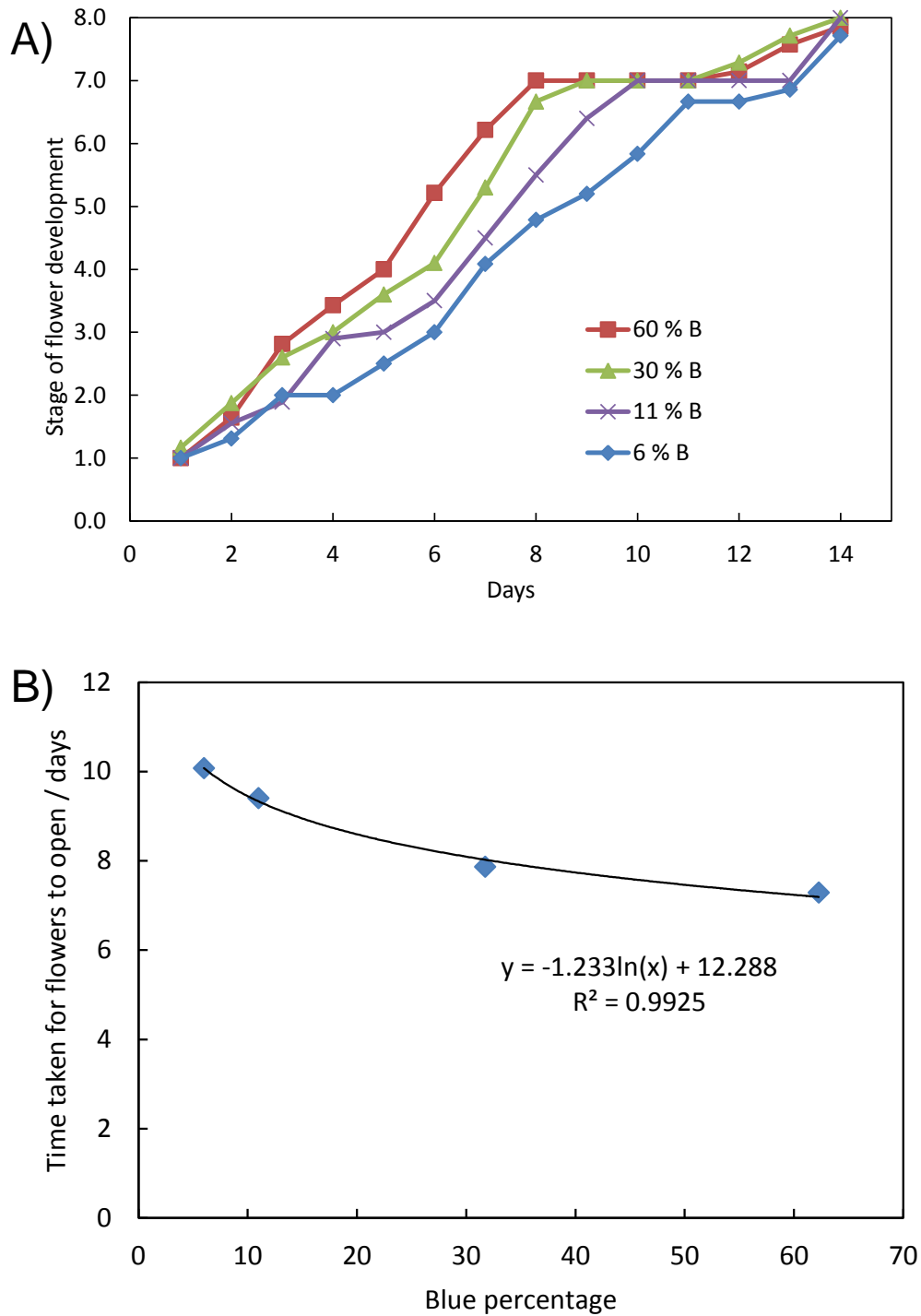


Figure 25. A) Time course of Petunia flower development for plants grown under light treatments with different red blue percentages. The 6% blue is a white light treatment. **B)** The influence of blue light percentage on the time taken for Petunia flower buds to open.

Discussion

WP 2.1b - Influence of red / blue ratio on plant growth

The Lettuce data presented here shows the data from three replicated experiments. The similarity of the results from the three replicates demonstrates that the LED light environment provides stable conditions that result in reproducible crop performance. Overall the findings are consistent and additive to the results reported in year one.

The greatest plant biomass was achieved under a light recipe containing between 10 and 15% blue light in the spectrum but that yields declined under 100% red light and as blue light percentage increased above 15%. The low yields under 100% red light are caused by the combined effects of leaf morphology and reduced stomatal conductance. Blue light is required to help leaves flatten out (uncurl) so under the 100% red light treatment the leaves remained curled which greatly reduces their ability to capture light and photosynthesize. Blue light is also important for opening stomata. If the stomata remain partially closed photosynthetic performance is limited by reduced access to CO₂ which enters the leaves through the stomata. For the vast majority of crop production systems some blue light must be included to maintain efficient photosynthesis and normal morphology. For light treatments with greater than 15% blue light the plant mass decreased due to changes in the efficiency with which plants can use the light for photosynthesis. Leaves use about 90% of the red light they absorb to drive photosynthesis, the remaining 10% is converted to heat. This efficiency drops to around 70% for blue light. As a result light use efficiency decreases as the blue light percentage increases. However, the measured biomass data is not observed to decrease linearly with increasing blue percentage because the leaf area also influences the total plant photosynthetic carbon gain and biomass. Leaf length and width were strongly influenced by light quality. Leaf length was shortest under 60% blue light and similarly long under 100% blue and 100% red light. This indicates that both red and blue light are important for maintaining plant compactness. Leaf width showed a similar response to light quality as leaf length but was also observed to decrease under 100% red light. It is possible that leaf width is influenced by resource availability (how much photosynthate has been produced) as well as light quality. The more compact plants were also observed to have thicker leaves (determined as leaf mass area : LMA) which indicates that plant resource partitioning was influenced by light quality rather than simply reducing the size of the leaves. Thicker leaves are expected to be more resilient to physical damage and potentially more resilient to pest and disease attack. These data can be used to design light recipes to create plants with the desired qualities for a specific purpose. For example robust compact plants may be most desirable for planting outside for field production while larger leaves may be more appropriate for hydroponic glasshouse production.

The two Lettuce varieties had similar responses to light quality though the absolute values of the measured parameters differed. There are many factors influencing plant habit and breeding programs have amplified natural variability to create plants with optimal qualities for specific uses. The Amica Lettuce is a summer variety while the Alega is a winter variety and so their lineages have been subject to different selection processes. With the data available it is not possible to explain the causes of the differences in the measured parameters between the varieties but there are several possible explanations including: different responses to temperature, different photosynthetic performance under these light treatments, different biomass partitioning, and different secondary metabolisms. Further experimentation would be necessary to begin to assess what factors caused the observed differences between the varieties. This information may be particularly useful for breeding programs focused on developing new varieties for LED lit controlled environment production systems (urban farms).

During the first year of this work we demonstrated that Petunia plants grown under 100% blue light flowered earlier and more extensively than those grown under red:blue mixtures. This year we have added our understanding of spectral effects on flowering by examining how light spectrum influences the speed at which flowers transition from buds to flowers and how long the flowers remain open. Flowers were observed to open most rapidly under 60% blue light, two days quicker than observed under white light containing 6% blue light. Plants were not grown under 100% blue light for the year two experiment because the morphology observed during year one was poor and unlikely to be recreated in commercial practice. Not only did flowers open more rapidly under higher blue light treatments they also remained open for a longer period. All flowers were observed to senesce after 14 days so flowers that opened two days earlier remained open for two days longer. High blue light or low red light treatments may be useful for maximising the numbers of open flowers on Petunia plants prior to sale.

Conclusions WP 2.1b - Influence of red / blue ratio on plant growth

Adjustment of the red:blue ratio can be used to manipulate crop morphology, however, such manipulation also affects growth rate. In general plant compactness increased as the blue percentage increased from 0 to 60% blue light. Plant mass, however, was observed to be greatest at 11% blue light and decrease as blue light percentage increased. The optimal spectral mix will potentially differ between species but will also depend on a grower's preference for rapid growth versus compact morphology.

Results WP 2.1c - Influence of red /far-red ratio on plant growth

Lettuce

Two Lettuce varieties (Alega and Amica) were grown under light treatments containing 11% blue light and 89% red light with and without additional far-red light treatments. The data presented show the results from three replicated experiments. The data from the three replicates were similar showing the consistency of plant quality from the LED4CROPS facility but also the robustness of the data set. Of the measured parameters the fresh mass was the most variable between replicates this is thought to be due to small differences in irrigation. For the Alega plants far-red light was found to result in a small increase in plants biomass (Figure 26A) but no influence was observed for the Amica plants. The numbers of leaves was found to decrease with increasing far-red light for Alega plants but not for the Amica plants (Figure 26B). Increasing far-red intensity resulted in increased leaf length in both varieties (Figure 26C) but had no influence on leaf width (Figure 26D) or leaf curling index (Figure 26E). Increasing far-red light was also found to result in decreases in leaf chlorophyll content (Figure 26F).

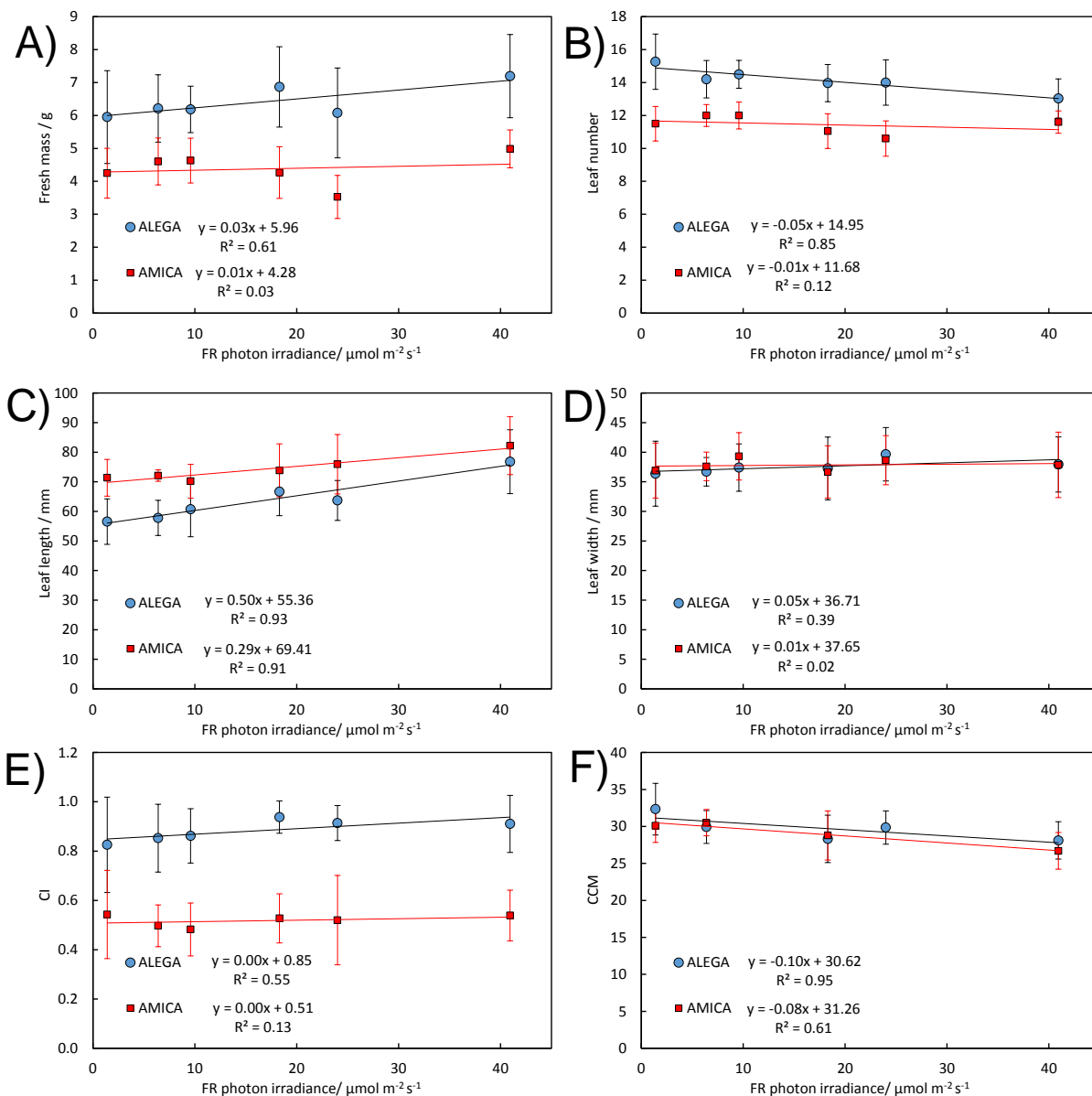


Figure 26. The influence of far-red light on growth and morphology of two Lettuce varieties, Amica and Alega. **A)** Shoot fresh mass collected after 21 days growth, **B)** mean number of leaves per plant, **C)** length of the forth leaf, **D)** width of the forth leaf, **E)** curling index (CI) and **F)** chlorophyll content measured using a CCM200 chlorophyll content meter. Error bars indicate standard deviation calculated using the measured values from all three replicates.

Discussion WP 2.1.c Influence of red : far-red ratio on plant growth.

The Lettuce results from this work package presented in the year one report represented data from one replicated experiment. The Lettuce data reported here represents the results from three replicates and include two additional far-red intensities. This replicated data set provides a more robust analysis of the influence of far-red light on Lettuce growth and morphology but largely provides the same results and conclusions as were presented in the year one report. Modern varieties of crop have undergone many years of artificial selection through breeding programs. These breeding programs have manipulated crop genetics to tune plant physiology so they can be grown in different seasons and/or have different morphologies. It is likely that during these breeding programs many of the genes associated with plant light responses have also been changed. The Lettuce varieties examined in these experiments have undergone different selection process and the Alega is a winter Lettuce variety while the Amica is a summer Lettuce variety. There is, therefore, the potential for these two varieties to have different light requirements and sensitivities in each region of the spectrum. The data reported here suggests that the two varieties have different responses to far-red light. Far-red responses are mediated by the phytochrome photoreceptors and are associated with shade avoidance syndrome and flowering initiation. In relation to Lettuce leaves red light is expected to activate the phytochromes reducing leaf length while far-red light is expected to inactivate the phytochromes thus increasing leaf length. The phytochromes sense both the intensity of a light source and also the red:far-red ratio of light. They regulate control of how long a leaf grows helping plants acclimate to their light environment. The length of Alega leaves were observed to increase more in response to the addition of far-red light than the Amica leaves. This difference is potentially caused by differences in sensitivity of the phytochrome mediated responses between the two varieties. In this case either, Alega is more sensitive to the addition of far-red light than Amica or Amica is less sensitive to the red light than Alega and so far-red light has less influence. If Amica's phytochrome responses are weaker than Amica's the differences may be associated with breeding aimed at reducing bolting in summer varieties. Phytochromes play an important role in regulating the transition from vegetative growth to reproductive growth. Any alterations in phytochrome signalling associated with changes in flowering time may also be reflected in plant morphology.

Conclusions WP 2.1.c - Influence of red : far-red ratio on plant growth.

Increasing the far-red intensity (reducing the red:far-red ratio) decreased plant compactness resulting in longer lettuce leaves. The responses of the two varieties differed slightly demonstrating the potential need to design light treatments with variety as well as species in mind.

Results WP 2.1d - Blue / red and far-red combinations

Lettuce

To examine how blue and far-red responses interact we grew two Lettuce varieties, Alega and Amica, under two red:blue ratios, (30% and 60% blue light, the treatments found to produce compact plants in WP 2.1b) with different amounts of additional far-red light (0, 11, ~20, ~35 $\mu\text{mol m}^{-2} \text{s}^{-1}$). During the experiment there was a fault on 60% blue no far-red treatment that resulted in the plants receiving a low dose of blue light through the night-period. This resulted in data that was not useable. However, because this treatment was included in the work package 2.1b the missing data could be replaced. While the light treatments were the same the plants were grown on different dates so where this data is presented it is identified by open symbols (Figures 27 and 28).

For Alega plants (Figure 27) far-red light was observed to increase shoot biomass for both the 60 and 30 % blue treatments. For the 60% blue treatments mass increased linearly with far-red intensity but for the 30% blue the greatest mass was observed for the 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ far-red treatment (Figure 27A). Leaf number was observed to decrease as far-red intensity increased for both the 30% and 60% blue light treatments though leaf number was larger in the 30% blue light treatment and decreased more rapidly with far-red than in the 60% blue treatments (Figure 27B). Leaf length of the 4th and 5th leaves (Figure 27C&D) increased linearly with increasing far-red intensity. The 60% blue treatments had a greater chlorophyll content than the 30% blue treatments. Far-red was observed to slightly reduce chlorophyll content (Figure 27E). Leaf width was observed to increase as far-red intensity increased for both 60 and 30% blue light treatments (Figure 27F).

For the Amica plants grown under 30% blue light fresh mass was observed to increase as far-red intensity increased (Figure 28A) with the greatest increases occurring between 0 and 11 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of far-red. For the plants grown under 60% blue light far-red resulted in a linear increase in fresh mass. For the Amica leaf number was not influenced by far-red intensity (Figure 28B). Far-red resulted in a linear increase in leaf length of both 30% and

60% blue treatments (Figure 28C&D) and in contrast to Alega this response was greatest for the 60% blue treatments. Chlorophyll content decreased with increasing far-red (Figure 28E). Far-red had little influence on the width of Amica leaves (Figure 28F).

In addition to varying the far-red dose in different red:blue backgrounds with the same total PAR photon irradiance we also examined the influence of far-red light on different photon irradiances of an 11% blue: 89% red light treatment for the Alega Lettuce variety (Figure 29). Far-red light was found to reduce leaf number in all but the highest

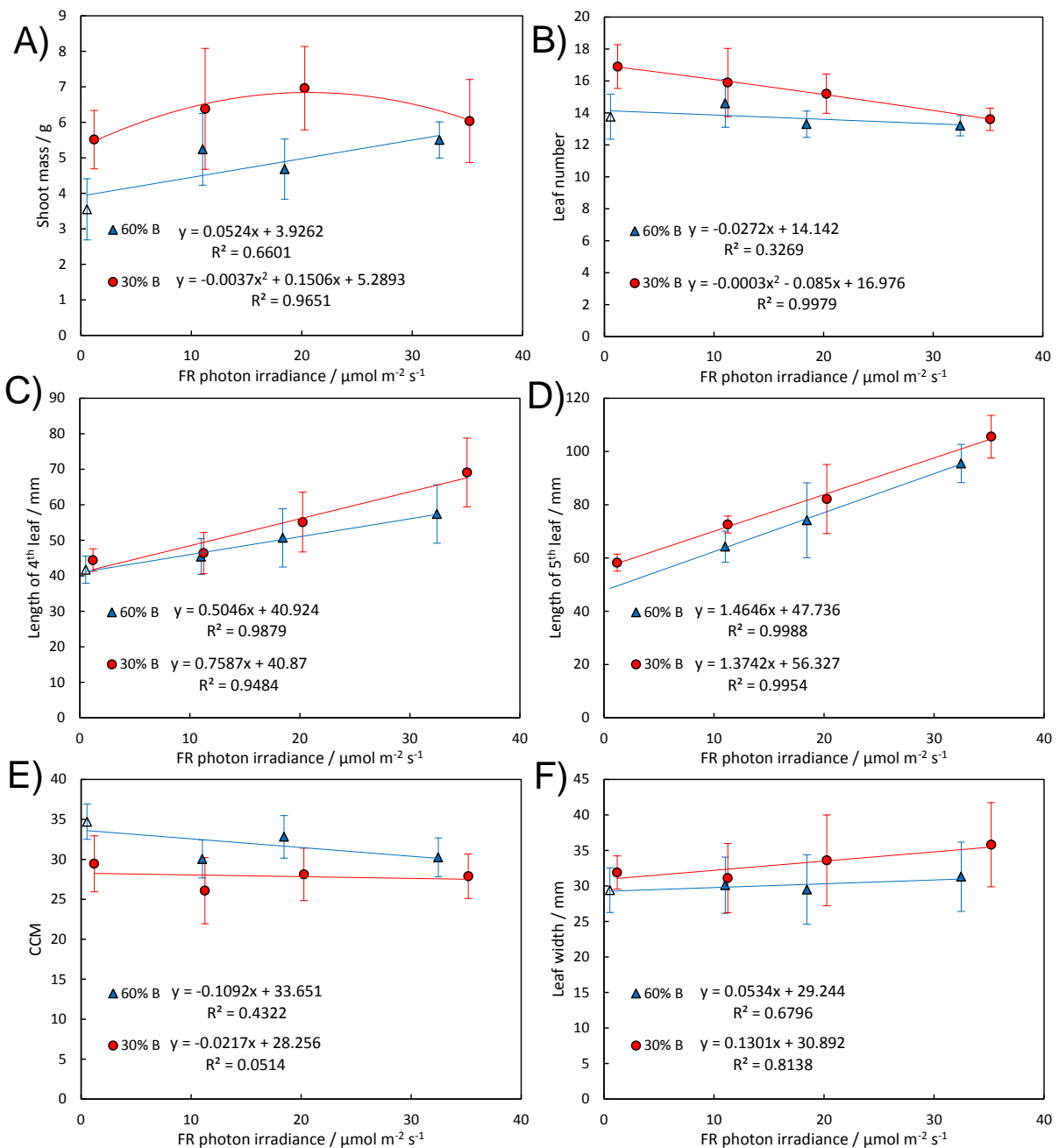


Figure 27. Influence of far-red on the **A)** mass, **B)** leaf number **C&D)** leaf length, **E)** chlorophyll content and **F)** leaf width of three week old Alega Lettuce plants grown under 30% blue and 60% blue light treatments. The open symbols indicate data taken from WP2.1b to replace values from the 60% blue with no far-red light treatment where a fault resulted in erroneous measured values.

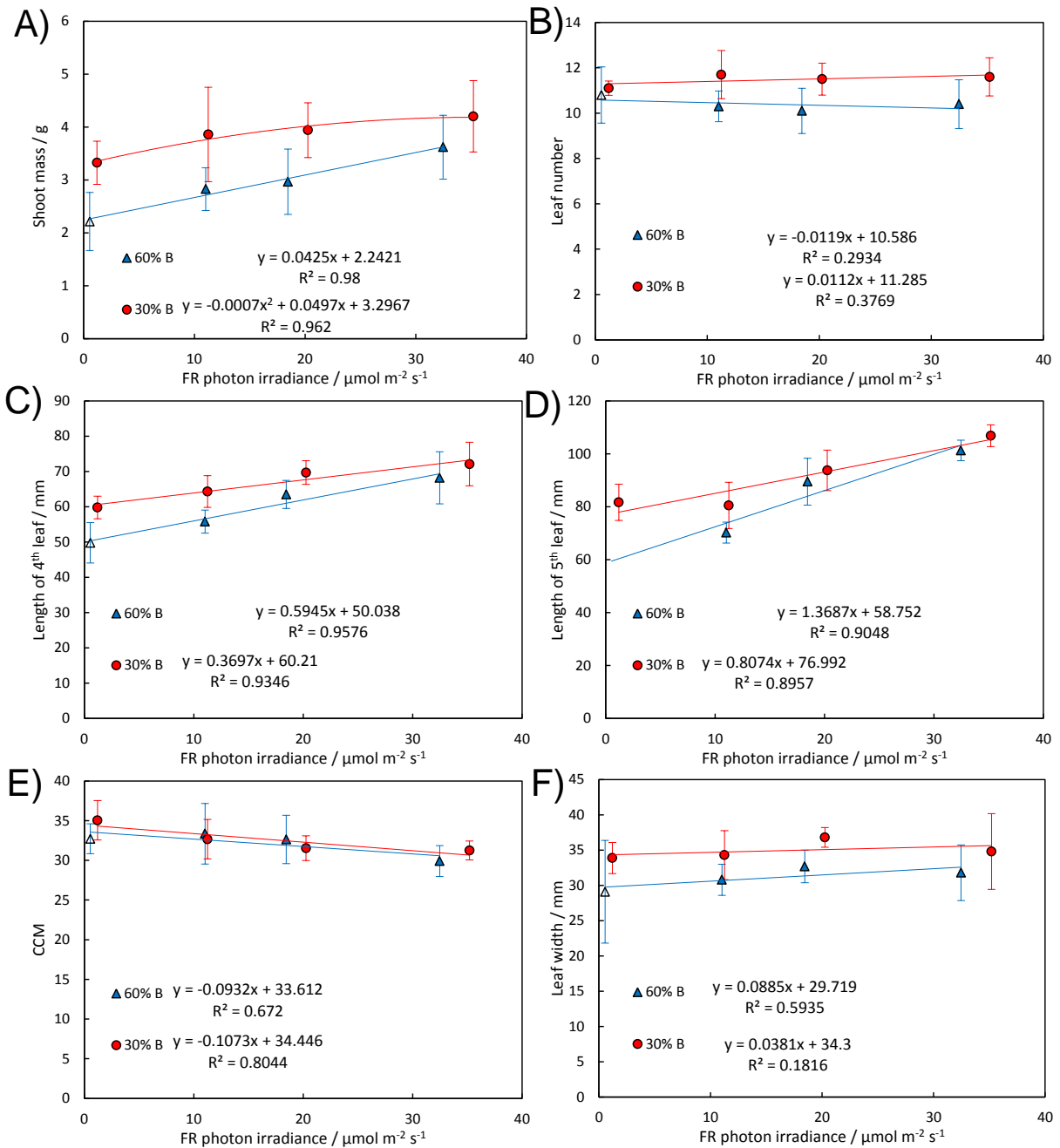


Figure 28. Influence of far-red on the **A)** fresh mass, **B)** leaf number **C&D)** leaf length, **E)** chlorophyll content and **F)** leaf width of three week old Amica Lettuce plants grown under 30% blue and 60% blue light treatments. The open symbols indicate data taken from WP2.1b to replace values from the 60% blue with no far-red light treatment where a fault resulted in erroneous measured values.

photon irradiance treatment ($360 \mu\text{mol m}^{-2} \text{s}^{-1}$; Figure 28A). The influence of far-red on fresh shoot mass was, however, small and PAR photon irradiance was the major controlling factor (Figure 28B). Leaf length was observed to decrease as light intensity increased and to be greater in the far-red treatments in all but the highest photon irradiance treatment (Figure 28C). For the no far-red treatment intensity did not influence leaf width (Figure 28D). However, in the far-red treatments leaf width was greatest at the two intermediate far-red intensities.

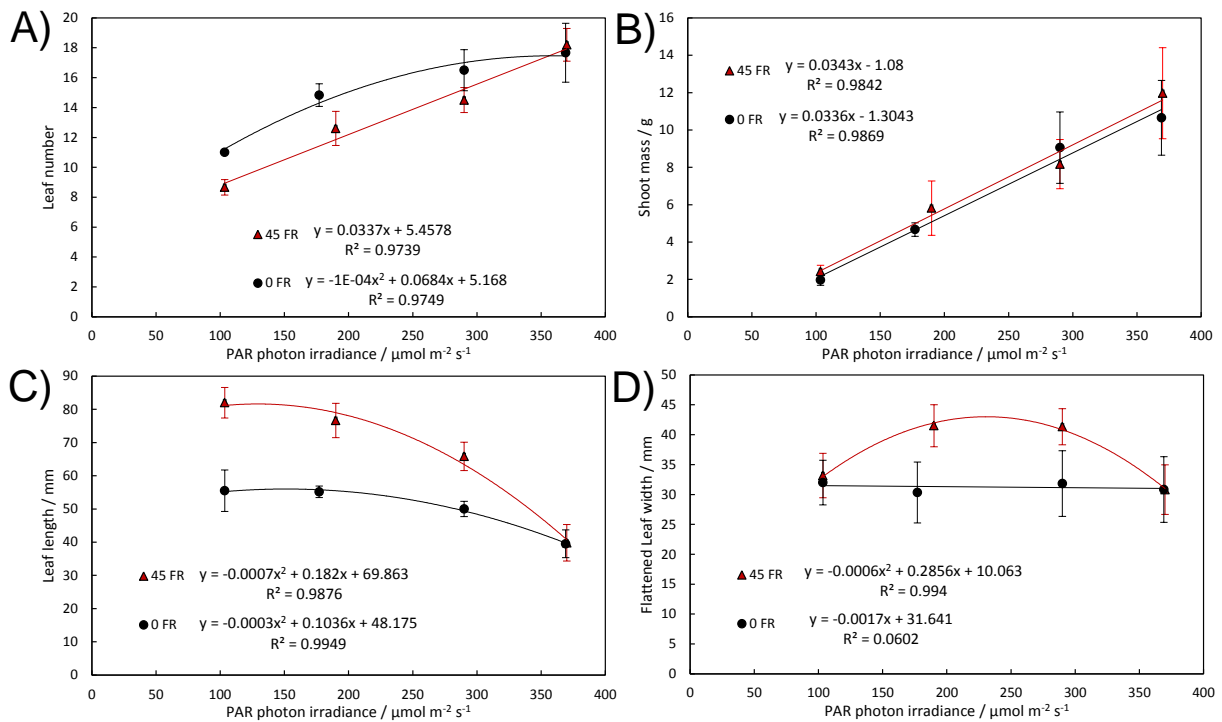


Figure 29. The influence of addition of far-red light to different photon irradiances of red:blue (89% R:11% B) light on Lettuce growth and morphology.

Petunia

Petunia plants were grown under eight different light treatments. Two blue percentages (30% blue and 60% blue) each with four different amounts of additional far-red light (0, 11, 20 & 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the 30% blue and 0, 11, 18 & 32 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the 60% blue). The plants grew well under all treatments and were ready for transplantation 26 days after sowing. While all treatments produced good quality plugs (Figure 30 and 31) there were noticeable differences in plant morphology. Far-red light produced plants with larger leaves.

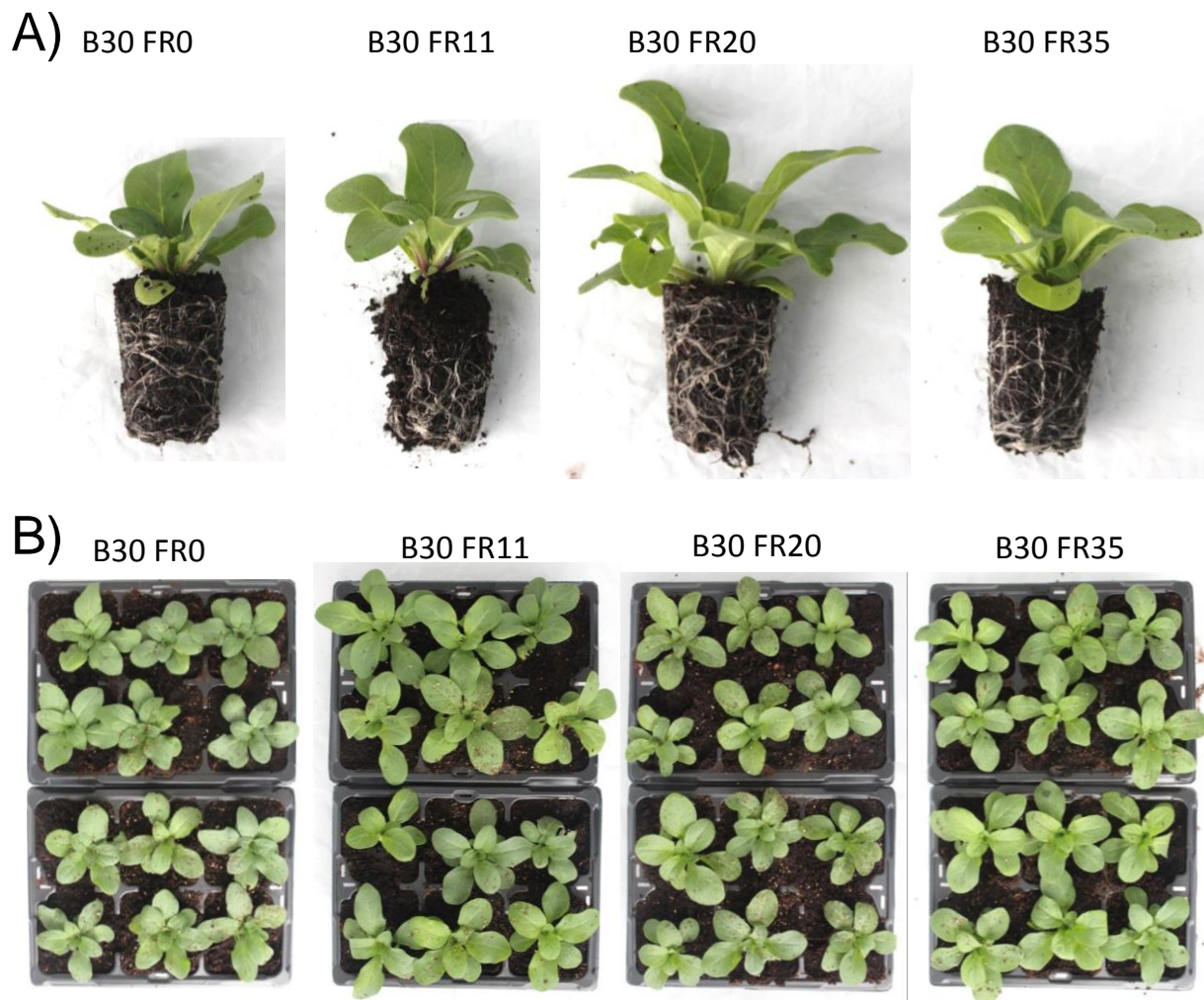


Figure 30. A) Petunia plug plants grown for 26 days (photographs taken on the 27th October 2015) under red blue light mixtures containing 30% blue light and different amounts of far-red (FR). **B)** Petunia plants immediately after transplanting to six-packs.



Figure 31. A) Petunia plug plants grown for 26 days (photographs taken on the 27th October 2015) under red blue light mixtures containing 60% blue light and different amounts of far-red (FR). **B)** Petunia plants immediately after transplanting to six-packs.

Destructive measurements of the plug plants identified the plants grown under 30% blue light to have higher biomass than those grown under 60% blue light (Figure 32). For the plants grown under 30% blue light an increase in far-red intensity from 0 to 11 $\mu\text{mol m}^{-2} \text{s}^{-1}$ increased biomass but further far-red increases reduced biomass. Far-red light had little influence on the biomass of plug plants grown under 60% blue light. Far-red light reduced the number of side shoots in plants grown under both 30% and 60% blue light: the 30% blue plants produced slightly more side shoots. Number of leaves per plant was observed to increase as far-red light intensity increased from 0 and 11 $\mu\text{mol m}^{-2} \text{s}^{-1}$, especially under the 60% blue light treatments, but decrease slightly at higher far-red intensities. Plants grown under 60% blue without far red contained more chlorophyll (measured using a chlorophyll content meter) and had darker green leaves than those grown under 30% blue and no far-red. The addition of far-red was observed to reduce chlorophyll content and this

effect was particularly pronounced in the 60% blue light treatments. Leaf lengths were greater under the 30% blue than the 60% blue light treatments. The increases in leaf length caused by far-red light occurred at lower far-red intensities under the 30% blue treatments than for the 60% blue light treatments. Far-red caused leaf width to decrease slightly in the 30% blue light treatments but to increase slightly under the 60% blue light treatments.

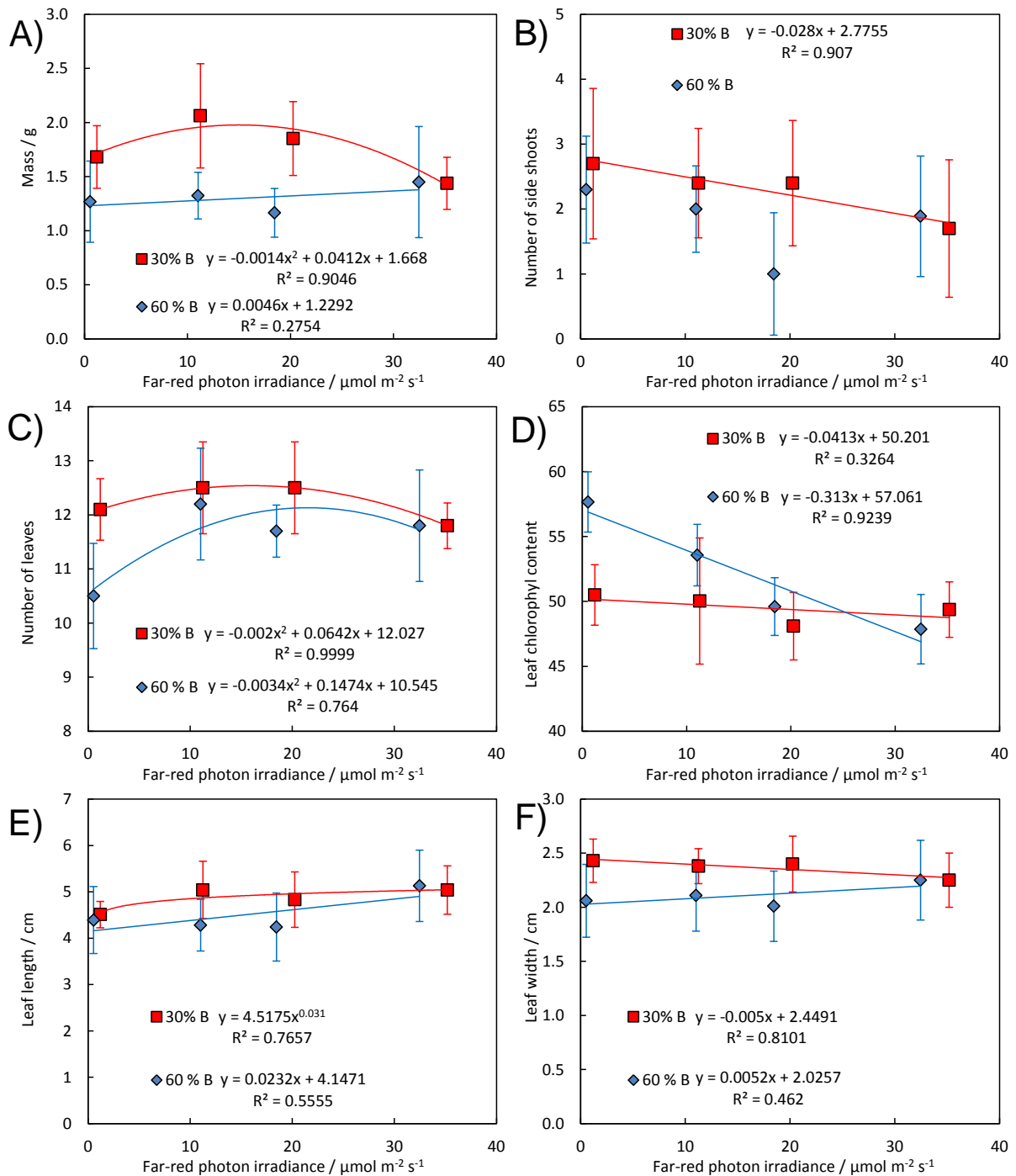


Figure 32. The influence of light quality on Petunia plug plant mass and morphology after 26 days growth under the different treatments.

Following transplantation the plants grew rapidly and nine days later the plant morphology was considerably different between the treatments (Figure 33). Plants were in full flower by the 13th November 2015 (17 days after transplantation and 43 days after sowing, Figure 34). Plant height and flower production were observed to increase with far-red light. The addition of far-red light had a more pronounced effect in the 60% blue treatments compared to the 30% blue light treatments.

Plant mass was observed to decrease as far-red intensity increased (Figure 35A). This response was more pronounced at lower far-red intensities in the 60% blue treatment than in the 30% blue light treatment. The number of side branches was greater in the 30% compared to the 60% blue light treatments. For both the 60% and 30% blue treatments increasing far-red from 0 to 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ resulted in a decrease in side shoots while further increases, increased the number of side shoots (Figure 35B). The length of the last vegetative leaf on the Petunias was unaffected by the light quality (Figure 35C). Leaf width was observed to increase with far-red light intensity, though for the 30% blue + 35 FR light treatment leaf width decreased (Figure 35D). Internodes increased linearly with far-red light intensity and were similar in length between the 30 and 60% blue plus far-red treatments (Figure 35E). Leaf robustness, assessed in proxy as leaf mass area (LMA), was found to be unaffected by the different light treatments (Figure 34F).

Flower bud development was affected by the different light treatments. Total number of flower buds was determined over a 25 day period (Figure 36A&B). Under both the 30% and 60% blue light treatments flower numbers were lower in the treatments containing no far-red light but were similar in the three treatments containing different amounts of far-red. Slightly more flowers were produced under the 30% blue light treatments. Number of open flowers was more strongly influenced by the amount of far-red provided than numbers of flower buds indicating that far-red light also influences flower opening (Figure 36C&D). The most open flowers were produced in the treatments with the most far-red light and the fewest open flowers were observed under the treatments with no far-red light. Numbers of open flowers were similar in the treatments containing 11-18 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of far-red light. Slightly more open flowers were produced under the 30% blue light treatments compared to the 60% blue treatments.

The flower development was assessed in the different treatments to determine the influence of far-red on opening speed (Figure 37). Under the 30% blue light treatments far-red light reduced the time it took a flower to open by only about half a day and most of this effect occurred between 0 and 11 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Under the 60% blue treatments flowers opened about one day quicker than those under the 30% blue treatments and the far-red light had a



Figure 33. Petunia morphology nine days after transplanting (5th November 2015), 35 days after sowing in the eight different red:blue:far-red light treatments.



Figure 34. Petunia plants grown under the eight red:blue:far-red light treatment. Plants photographed on the 13th November 2015, 47 days after sowing.

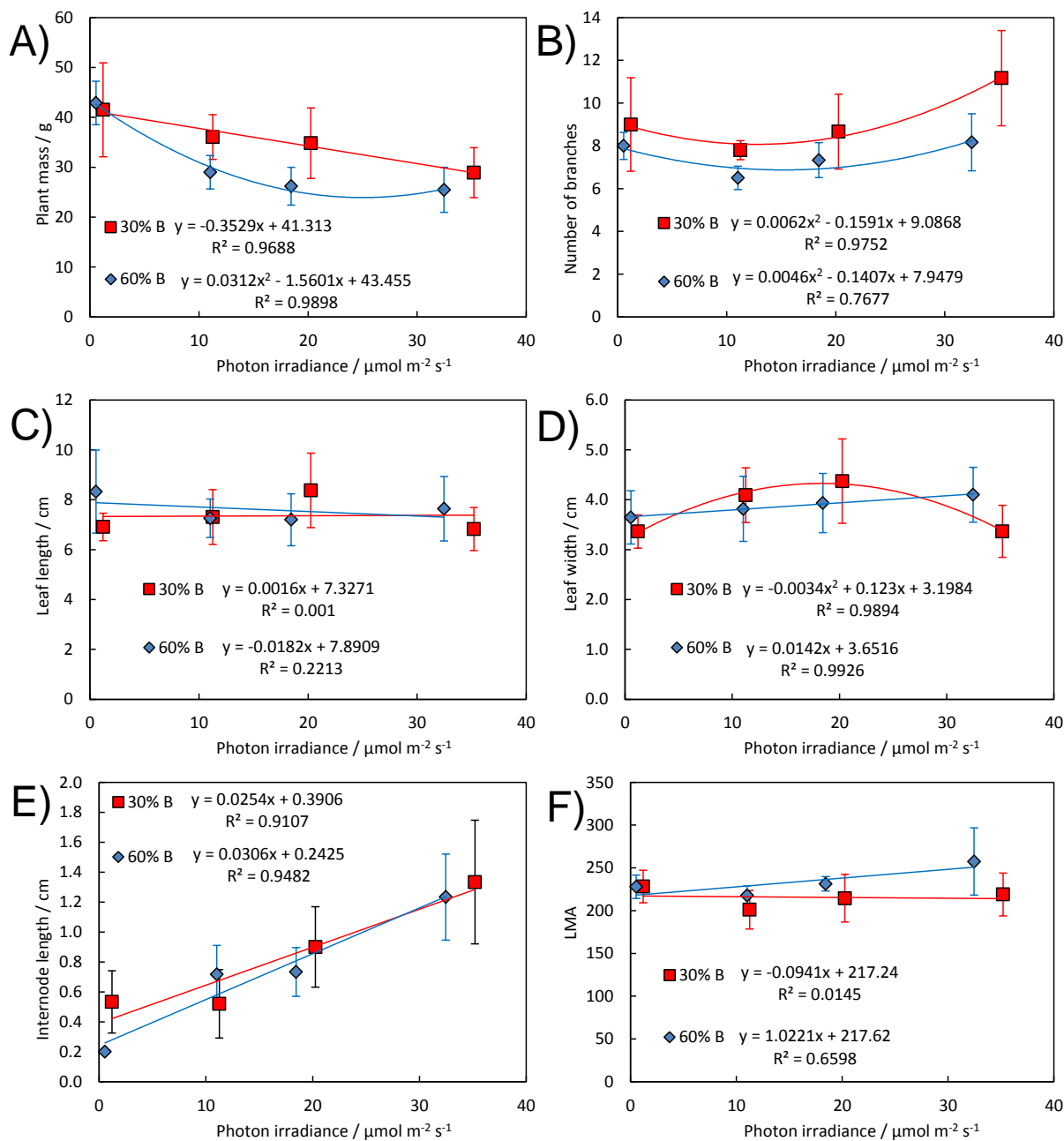


Figure 35. Influence of red, blue and far-red light on *Petunia* plant mass and morphology. Blue symbols and lines represent data from plants grown under 60% blue + 40% red light with different amounts of far-red. Red symbols and lines represent data from plants grown under 30% blue + 70% red light with different amounts of far-red. **A)** Plant mass, **B)** number of branches longer than 1 cm, **C)** length and **D)** width of the last vegetative leaf, **E)** length of the internode below the last vegetative leaf and **F)** leaf mass area (LMA) of the last vegetative leaf.

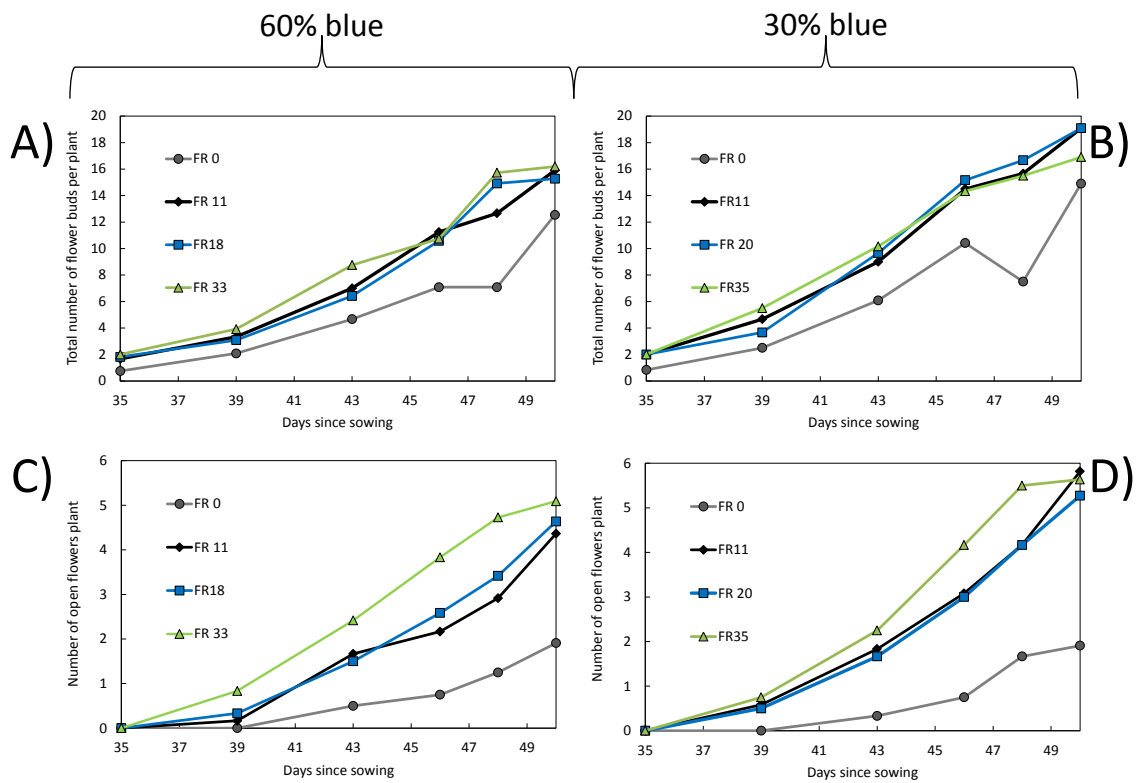


Figure 36. The influence of far-red light on the time course of Petunia flower production under **A)** 60% blue and **B)** 30% blue light. The influence of far-red light on the time course of number of open flowers under **C)** 60% blue and **D)** 30% blue light.

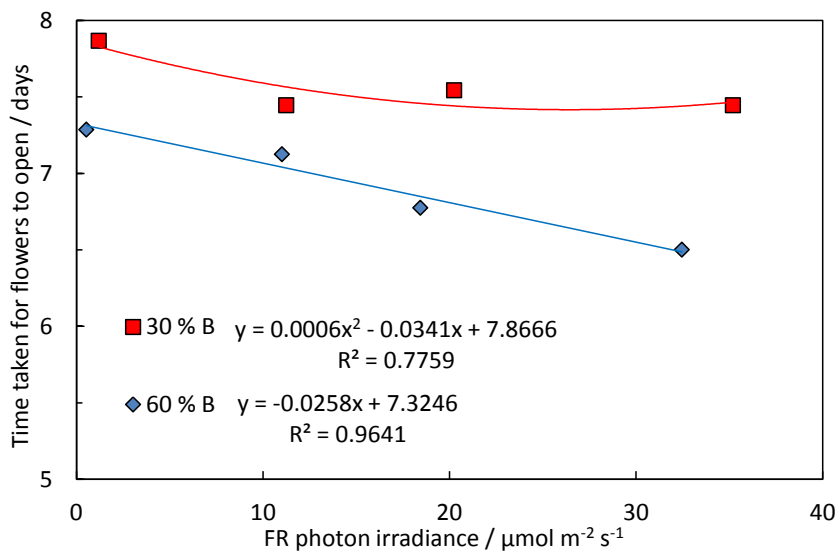


Figure 37. The influence of blue and far-red light on the time it takes Petunia flowers to open.

greater influence on opening speed. $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ of far-red light reduced opening speed by 1 day. Flower morphology was also altered by light quality (Figure 38 and 39). Flower diameter was found to increase with far-red intensity. Flowers from the 30% blue treatment were slightly larger than those from the 60% blue treatment but the influence of far-red light was similar for both sets of blue light treatment. While far-red was found to increase flower size the sepal size was found to decrease as far-red intensity increased.



Figure 38. Photographs of Petunia flowers grown under the eight different red:blue:far-red light treatments.

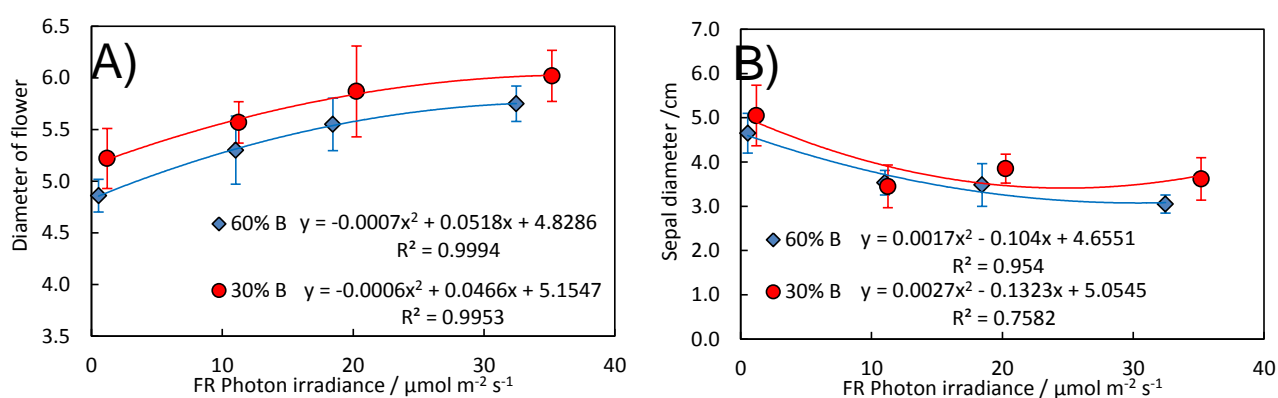
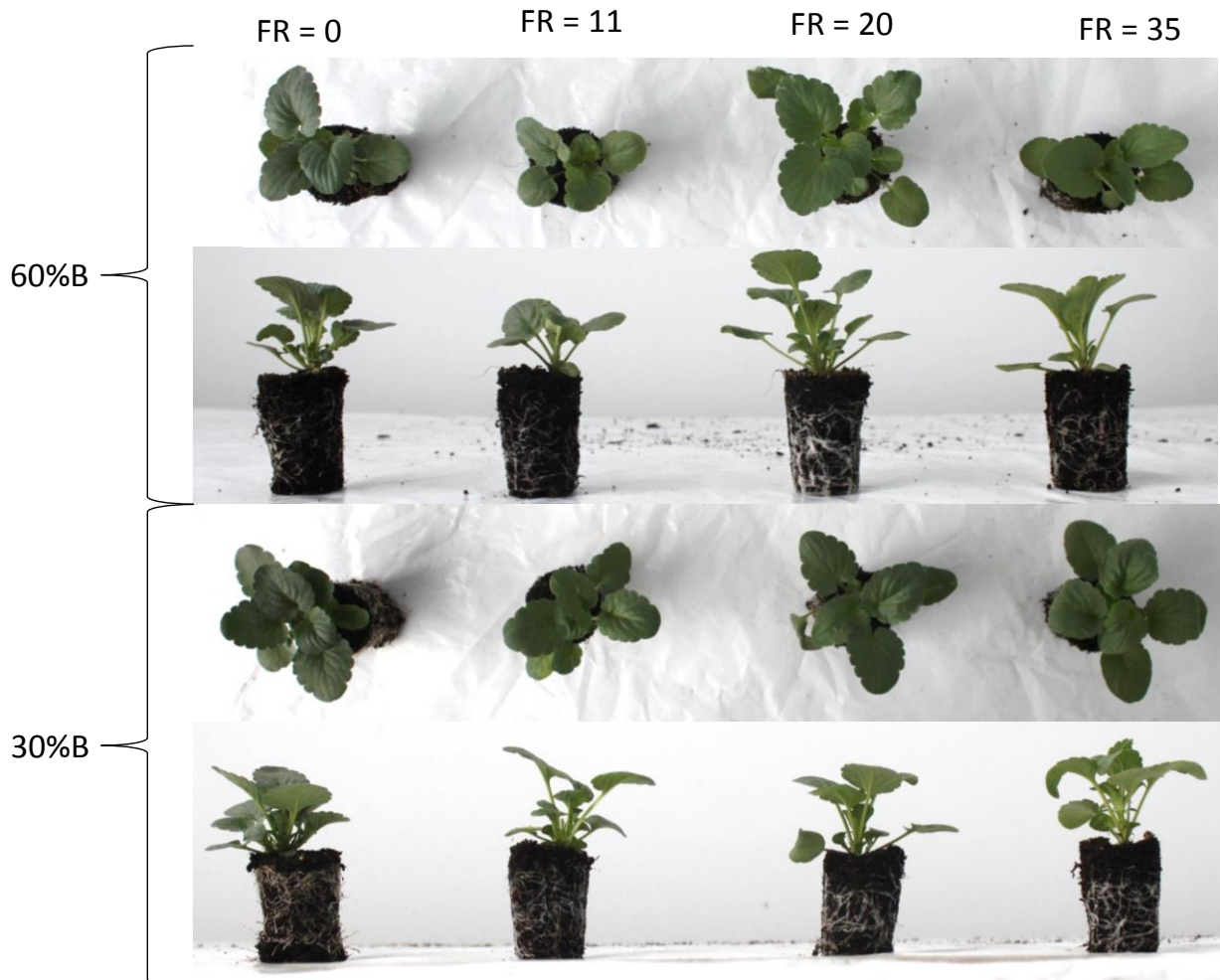


Figure 39. The influence of light quality on Petunia flower diameter **A)** and sepal length **B)**.

Pansy

Pansy plug plants were grown under the same eight target light treatments as the Petunia plants (Figure 40). The plants were similar in appearance between the treatments, though the plants grown in the absence of far-red light had shorter petioles and a more compact appearance. Pansy plug plants were ready for transplantation 40 days (10th November 2015) after sowing. Root development was good in all treatments and the plugs held together during handling.

Figure 40. Pansy plug plants from the eight light treatments on 10th October 2015, 40 days after sowing.



Following transplantation, the Pansies grew rapidly and flower buds were visible in some of the treatments within one week. The differences in light treatment became more pronounced as the plants grew (Figure 41).

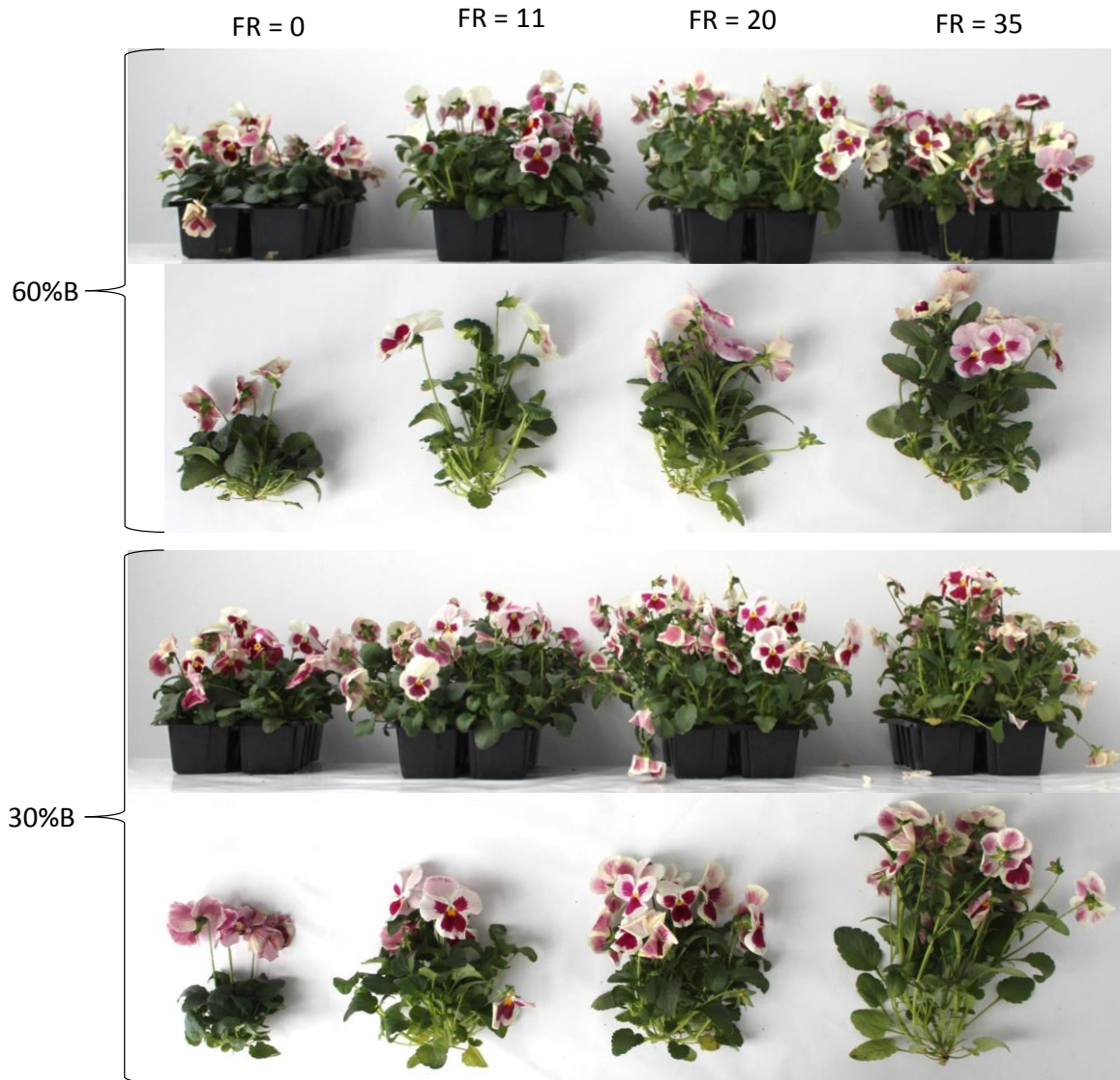


Figure 41. Pansy plants photographed on the 14th December 2016. 105 days after sowing.

The addition of far-red light to the spectrum was found to increase fresh mass (Figure 42A). In the 30% blue treatment the greatest mass was associated with the highest far-red intensity ($35 \mu\text{mol m}^{-2} \text{s}^{-1}$) but in the 60% blue treatments the fresh mass was observed to decrease between the 20 and $34 \mu\text{mol m}^{-2} \text{s}^{-1}$ far-red treatments. The number of side branches (Figure 42B) was found to decrease as far-red intensity increased. This response was greatest in the 60% blue light treatment. Internode lengths (Figure 42C) were observed to increase with the addition of far-red in the light spectrum, again this response

was more pronounced in the 60% blue than the 30% blue treatment. Leaf length (Figure 42D) was observed to increase between 0 and 11 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for both the 30% and 60% blue light treatments. Further increases in far-red had no effect on leaf length. Leaf width (Figure 42E) was largely unaffected by the amount of far-red light in the spectrum. Chlorophyll content (Figure 42F) was observed to decrease as far-red intensity increased.

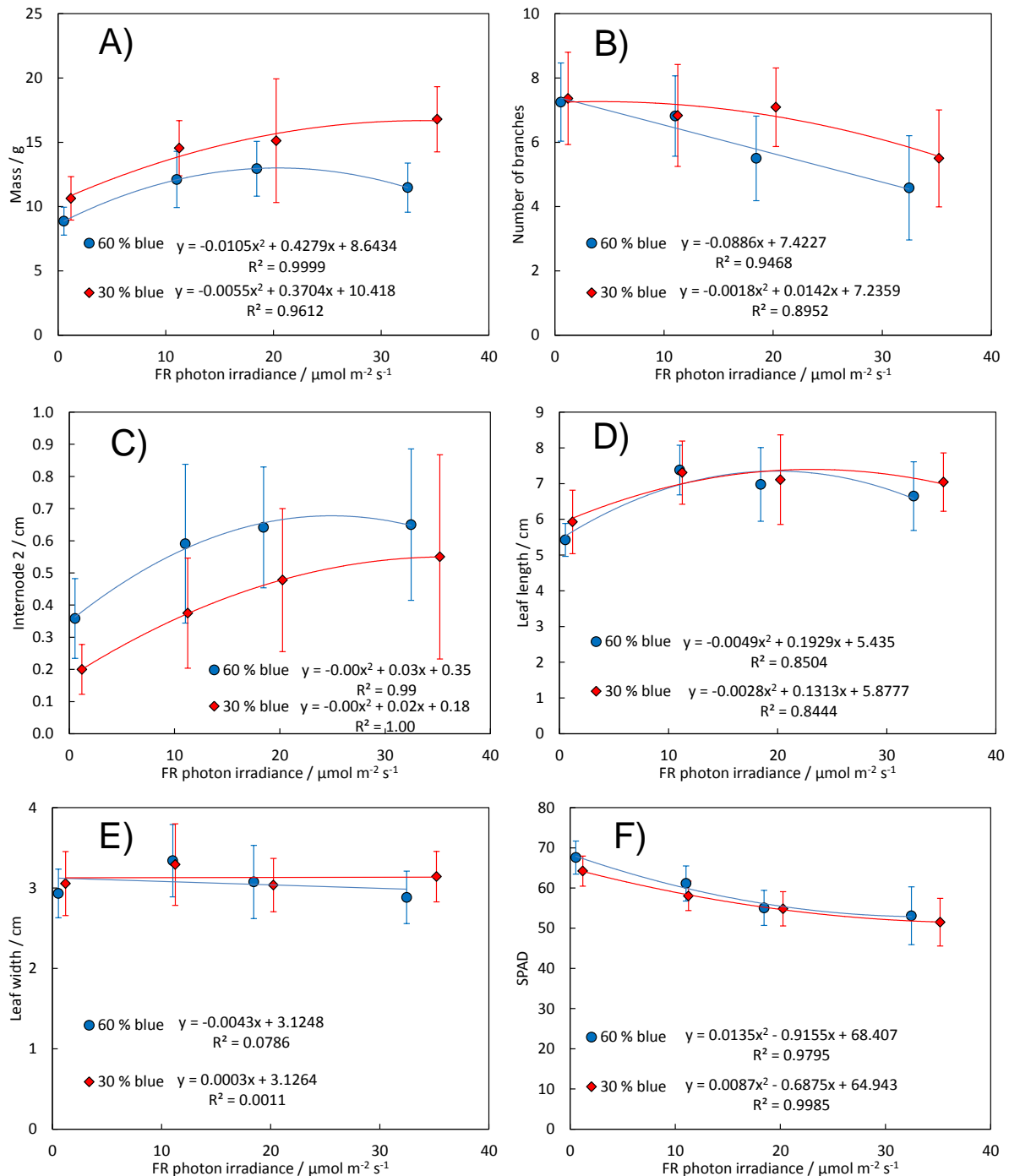


Figure 42. The influence of far-red light intensity on the growth and morphology of Pansy plants grown under light spectra containing either 30% or 60% blue light. Plants were assessed 105 days after sowing.

Pansy flowering was influenced by both blue light percentage and far-red intensity (Figure 43). The number of flower buds produced per plant was greater in the 30% blue than the 60% blue light treatments and this correlated with differences in plant mass (larger plants produced more buds). Numbers of flower buds were also observed to increase as far-red intensity increased. This was partially explained by differences in plant mass caused by far-red light but also partially by a far-red promotion of flower production. The highest far-red intensity treatments advanced flower bud production by approximately 5 days compared to the no far-red treatments. Far-red intensity also had a pronounced influence on flower opening. Flowering was advanced by 15 days in the highest far-red treatments compared with the no far-red treatments. Under the 60% blue light treatments less far-red light was required to achieve maximum flowering than in the 30% blue light treatments.

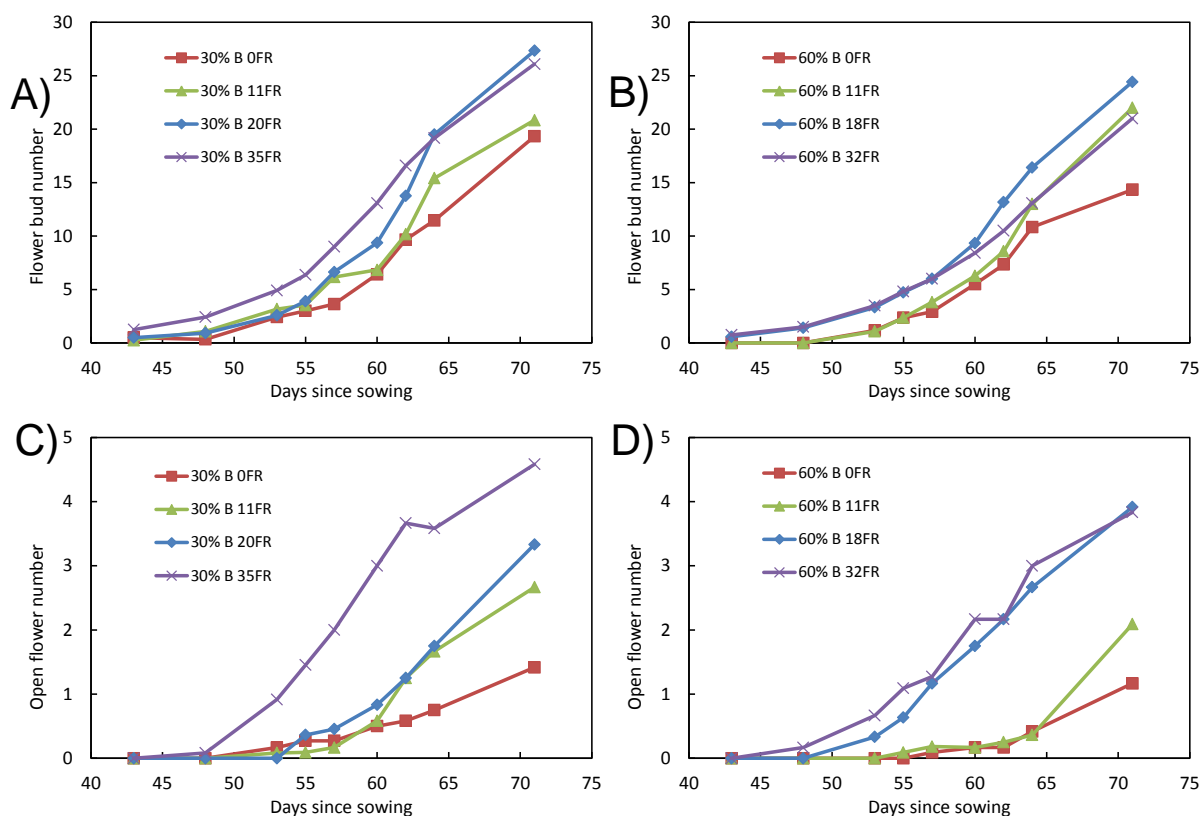


Figure 43. The influence of different combinations of blue, red and far-red light on the time course of number of flower buds (A & B) and open flowers (C & D) produced by Pansies. Graphs A) and C) show the data for plants grown in a 30:70 blue:red light mixture with different amounts of far-red light and graphs B) and D) show the data for plants grown in a 60:40 blue:red light mixture with different amounts of far-red light.

Discussion WP 2.1d - Blue / red and far-red combinations

The data presented in work package 2.1b in this report and in the year one report demonstrates that light treatments with between 30% and 60% blue light resulted in the most compact plants. The increased plant compactness is, however, associated with a reduced growth rate probably due to 1) a lower light use efficiency (blue light is used less efficiently for photosynthesis than red light) and 2) reduced leaf area and a corresponding lower light interception. Slower growth rates will result in slower flowering speeds which could delay plant sale. The results from work package 2.1c demonstrated that adding far-red light to a mixture containing 11% blue light increased flowering speed by up to two weeks compared to treatments with no far-red light. However, far-red light resulted in reduced plant compactness, with increased internode lengths and reduced leaf pigmentation. This work package (WP 2.1d) was designed to examine whether combining high blue light percentages with far-red light treatments would allow the development of light treatments that combine the benefits of high blue (compact plants) and far-red (early flowering) light to produce the ideal plants without the need for plant growth regulators.

Overall the plants grown in the WP 2.1c experiments responded consistently with the data from previous trials. High blue (30% and 60% blue) percentages with no far-red resulted in compact plants and the plants grown under 30% blue grew more rapidly than those grown under 60% blue light. The inclusion of far-red light in the spectrum resulted in plant stretching and reduced pigmentation. The positive influence of far-red light on flowering speed remained with Pansy flowers opening up to 15 days earlier and Petunia flowers opening one week earlier, compared with the no far-red treatments. There was no evidence from these experiments that blue light reduced the influence of far-red light on plant morphology. This is consistent with our understanding of the photoreceptor signalling pathways in which blue light responses and red:far-red light responses function via separate pathways. It is, however, also the case that far-red does not remove the influence of blue light treatments that produced the most compact plants. So compact plants (high blue light treatments) treated with far-red to induce flowering will become less compact but will still remain more compact than plants grown under light treatments producing less compact plants (low blue treatments). These data indicate that with further light recipe development red:blue:far-red treatments could be used to produce compact rapidly flowering plants. For example it may be possible to identify a lower far-red light intensity that is sufficient to induce flowering but has less effect of morphology. There is likely an optimum balance between achieving the desired compact morphology and speed of flowering. Comparison of the plants grown under the 30% and 60% blue light treatments in these experiments

indicates that the 30% blue light treatment produced the best plants. This is because 30% blue produced plants with greater biomass that were equally or more compact. In addition to producing equal or better quality plants with larger flowers the 30% blue light spectrum is more energy efficient.

While overall the Pansy and Petunia plants responded similarly to the different light treatments there were a few examples where their responses differed considerably. The addition of far-red light was observed to reduce the mass of the Petunias but to increase the mass of the Pansies. One possible explanation for these differences may be how the light influences resource use in the different species. The far-red treatments resulted in more rapid and extensive flowering in the Petunia but none of the resources put into the flowers and scents were recorded in these experiments. These un-measured resources may result in the apparent decrease in biomass with increased far-red treatment in Petunias. At the point when the Pansy biomass was measured the flowering was at an early stage and so fewer resources had been 'lost' due to turnover of flowers. The data on numbers of side shoots also different between the two species. Numbers of side shoots decrease as far-red intensity increased in Pansy. In Petunia, however, the number of side shoots was observed to decrease up to $11\mu\text{mol m}^{-2} \text{s}^{-1}$ far-red and then to increase as far-red intensity increased further. In Pansy the decrease in side shoots is associated with a greater investment in the primary shoots with large increases in internode lengths and reduced investment in side shoots. In Petunia the far-red was also observed to cause stretching of the side shoots. In this experiment side shoots were counted when they were greater than one centimetre in length. Any stretch of these side shoots would therefore be expected to result in an increase in shoot counts. It is thought that the increased numbers of side shoot in the Petunia is a result of the counting artefact and that the numbers of shoots remained the same.

These results, while positive, did not identify a light treatment that could increase flowering with no influence on other plant qualities. This goal may still be achievable if we explore the possibility of providing light treatments specific to different stages of crop growth. For example plants could be grown with no far-red until flowers are required. At this point far-red treatments could be applied to induce flowering. Once flowering has been induced the far-red treatments could be removed to reduce ongoing plant stretching. Once induced flowering may continue without further exposure to far-red light allowing new growth to remain compact.

Conclusions WP 2.1d - Blue / red and far-red combinations

Light treatments combining different red:blue and red:far-red ratios were examined with the aim of producing compact plants that flower rapidly. These experiments indicate that the influence of far-red and blue light on morphology function independently. This means that the influence of high blue percentages can not remove the stretching induced by the far-red light that can be used to hasten flowering. Also in many cases the influence of far-red light was greater under 60% blue than 30% blue light. This was because the red:far-red ratio was lower in the 60% blue treatments. However, careful design of the light spectrum will enable production of high quality plants with good morphology and advanced flowering. Furthermore by providing different light treatments at different stages of growth it is expected that greater control of morphology and flowering time will be possible.

Results WP 2.3 - Improving HNS Propagation

Santolina 'Lemon Fizz'

At the Kernock Park Plants site 2015-2016 was a low light year and this provided a good test of the benefits of the different pre-treatments for cutting production. The three different pre-excision treatments resulted in large differences in the total number of cuttings available. The supplemental ($51\mu\text{mol m}^{-2} \text{s}^{-1}$ of light for 12 hours) pre-excision treatment provided the most cuttings (490) followed by the unlit pre-treatment (300) and the fewest were provided by the day-length extension treatment (200). Overall appearance and quality of the cuttings collected from the three supplemental pre-excision treatments also differed. The best quality cuttings were observed from the supplemental treatment with those from the unlit treatment close in quality. The day-length extension pre-excision treatment cuttings were generally the weaker and first to show signs of stress.

Following sticking the cuttings were placed under the different LED light treatments. The cuttings that remained healthy retained a green stem and leaves. As plants became stressed the leaves wilted and turned brown. Plants were regarded as dead when the stem (not the leaves) had turned brown (Figure 44). In most cases when the plants had died a large fungal mass developed from the stem outwards. It was not clear whether the fungus was the cause of the death or a secondary infection.

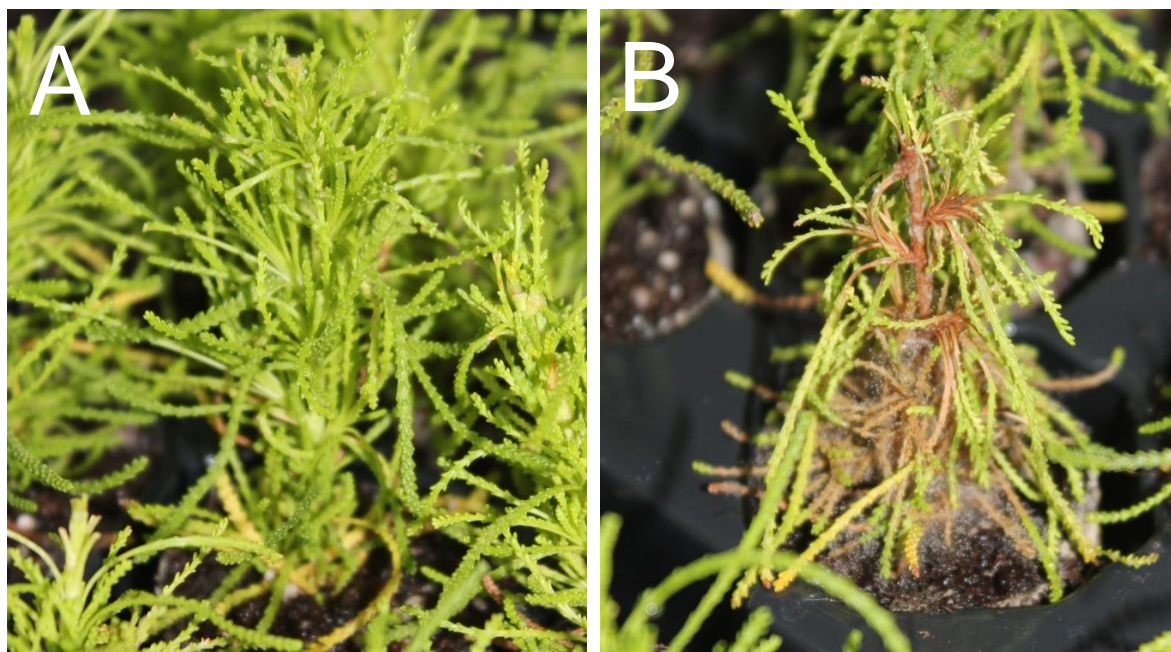


Figure 44. Photographs of **A)** a healthy *Santolina* cutting and **B)** a dying *Santolina* cutting. Note that on the dying cutting the stem is turning brown first and that many of the leaves remain healthy looking at their tips.

The influence of pre-treatment on Santolina cutting rooting

The general health of the cuttings from the three pre-excision treatments was similar and good when they were received from Kernock Park Plants though the cuttings from the day-length extension treatment were a slightly paler shade of green than those from the other two treatments. The influence of pre-excision treatment on cutting survival and rooting, determined from the four treatments where cuttings from all pre-excision treatments were available (see Table 9), is shown in Figure 45. Cutting survival was 80% for the supplemental pre-excision treatment, 67% for the unlit pre-excision treatment and 52% for the day-length extension pre-excision treatment. The percentage of cuttings that rooted and the survival corrected percentage of cuttings that rooted followed the same trend, with the greatest success associated with the supplemental pre-excision treatment (67.2% rooted) and the worst performance associated with the day-length extension pre-excision treatment (33% rooted).

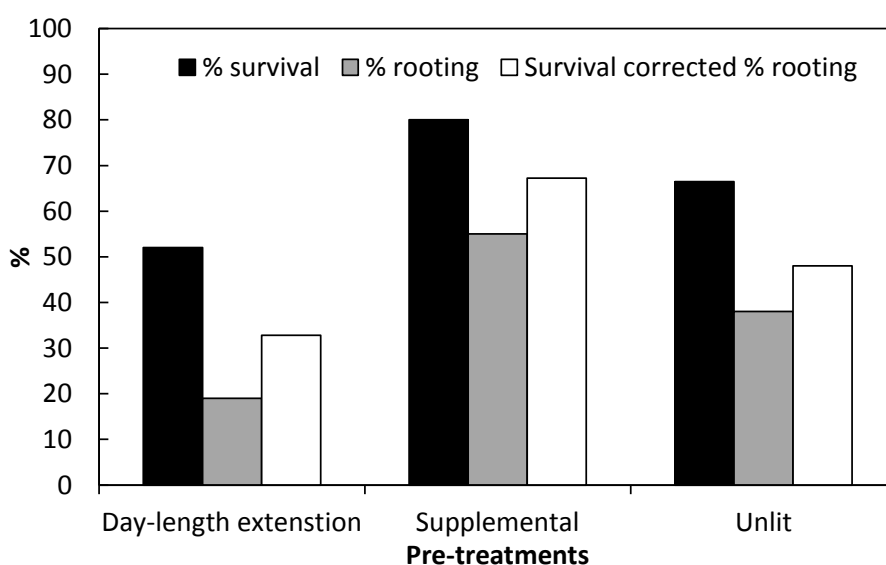


Figure 45. The influence of pre-excision treatment provided to the mother stock plants on the percentage of survival and rooting for the Santolina cuttings. Values were calculated using data from the four light treatments where cuttings from all three pre-excision treatments were present, see Table 9.

Influence of pre-excision treatment and blue light intensity on Santolina cutting rooting

Following exposure to the red-blue LED treatments differences in the cutting responses between both treatment and pre-treatment were visible in the plant material. After 11 days exposure to the treatments (Figure 46) plant stress (browning of the leaves) was observed to increase with blue light from 100% red light through to the 60% blue light treatment.

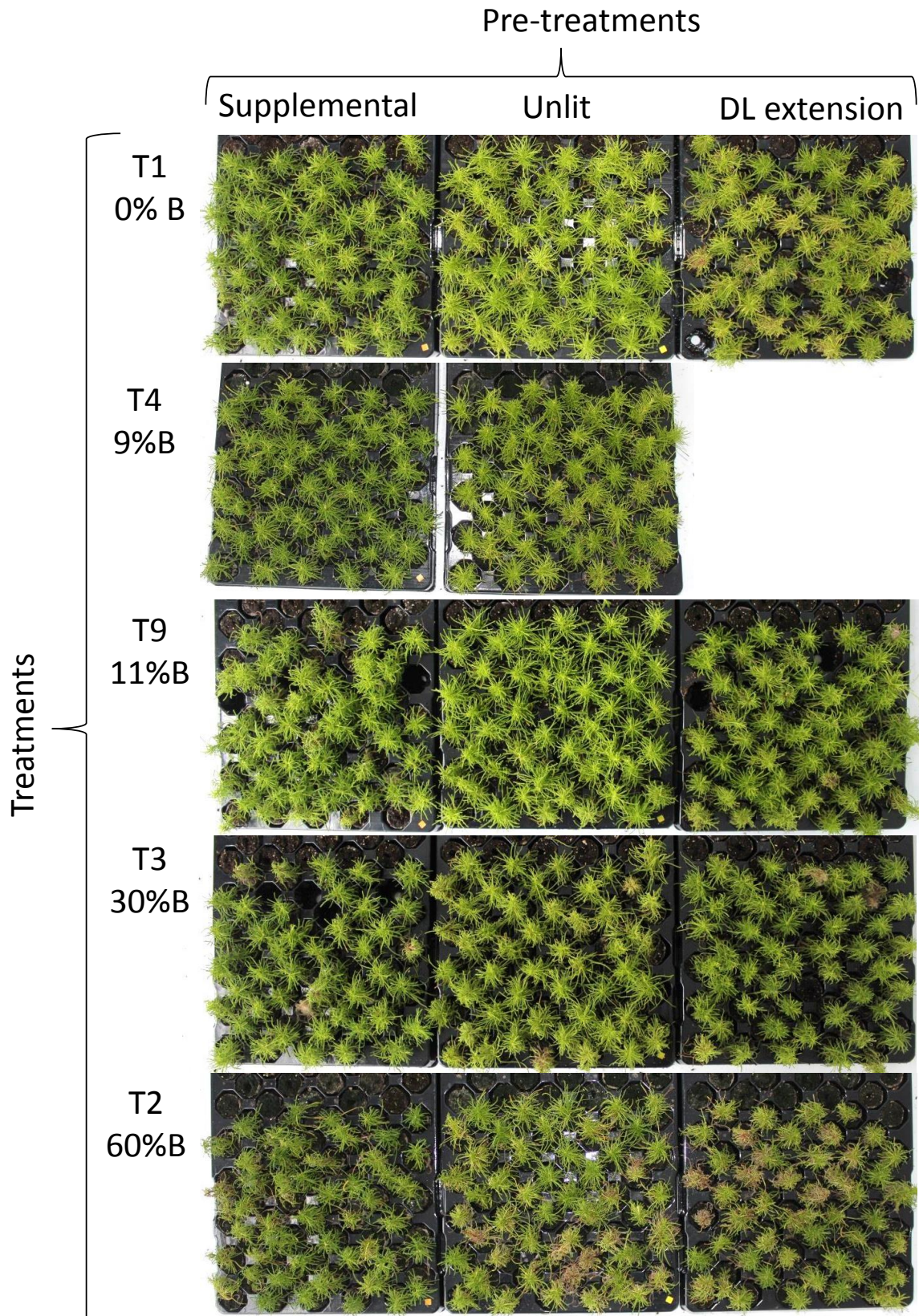


Figure 46. Influence of pre-excision treatments and blue percentage of light-treatments on the appearance of Santolina cuttings after 11 days (2nd February 2016) in the LED4CROPS facility.

Plants from the supplemental pre-excision treatment showed the least signs of stress and those from the day-length extension treatments showed the greatest levels of stress. After 27 days exposure to the treatments, pre-excision treatment effects had become pronounced (Figure 47) and in treatments where higher levels of stress (higher blue percentage treatments, unlit and day-length extension pre-excision treatments) were observed many cuttings had died and been removed. The greatest influence of pre-excision treatment was observed in the 60% blue treatment where the majority of the supplemental pre-excision treatment plants remained green (though they were showing early signs of water deficits) while many of the plants from the other pre-excision treatments were dead or dying. The benefits of the 100% red light treatment were also highlighted by the fact that many of the plants from the day-length extension pre-excision treatment (the worst performing treatment) remained green.

The blue light percentage of the light treatment was found to have a strong influence on cutting survival and rooting success (Figure 48). Cutting survival of the supplemental pre-excision treatment cuttings was observed to be independent of the blue percentage with over 90% survival even in the 60% blue light treatment. However, survival was observed to be lower in the 11% and 30% blue light treatments (60% and 68% survival respectively). The cause of the poor performance of the cuttings in these treatments was thought to be due to a higher disease pressure. Survival of the unlit and day-length extension pre-excision treatments was found to decrease markedly as the blue intensity was increased from 0 to 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

To assess the number of cuttings that rooted we determined the survival corrected rooting percentage (Figure 48B). This calculation partially removes the variation in rooting between treatments that is caused by disease or dehydration and helps to reveal photobiological effects on rooting. Cutting rooting was found to decrease as the blue light intensity increased from 0 to 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This effect was strongest for the unlit pre-excision treatment cuttings. Rooting was particularly poor for the day-length extension pre-excision treatment where rooting percentage was below 50% even in the 100% red light treatment. The number of roots produced per cutting was also found to decrease as blue light intensity increased though the pre-excision treatments effects were less pronounced (Figure 48C). Root length was found to be influenced by neither pre-excision treatment nor blue percentage of the light treatment.

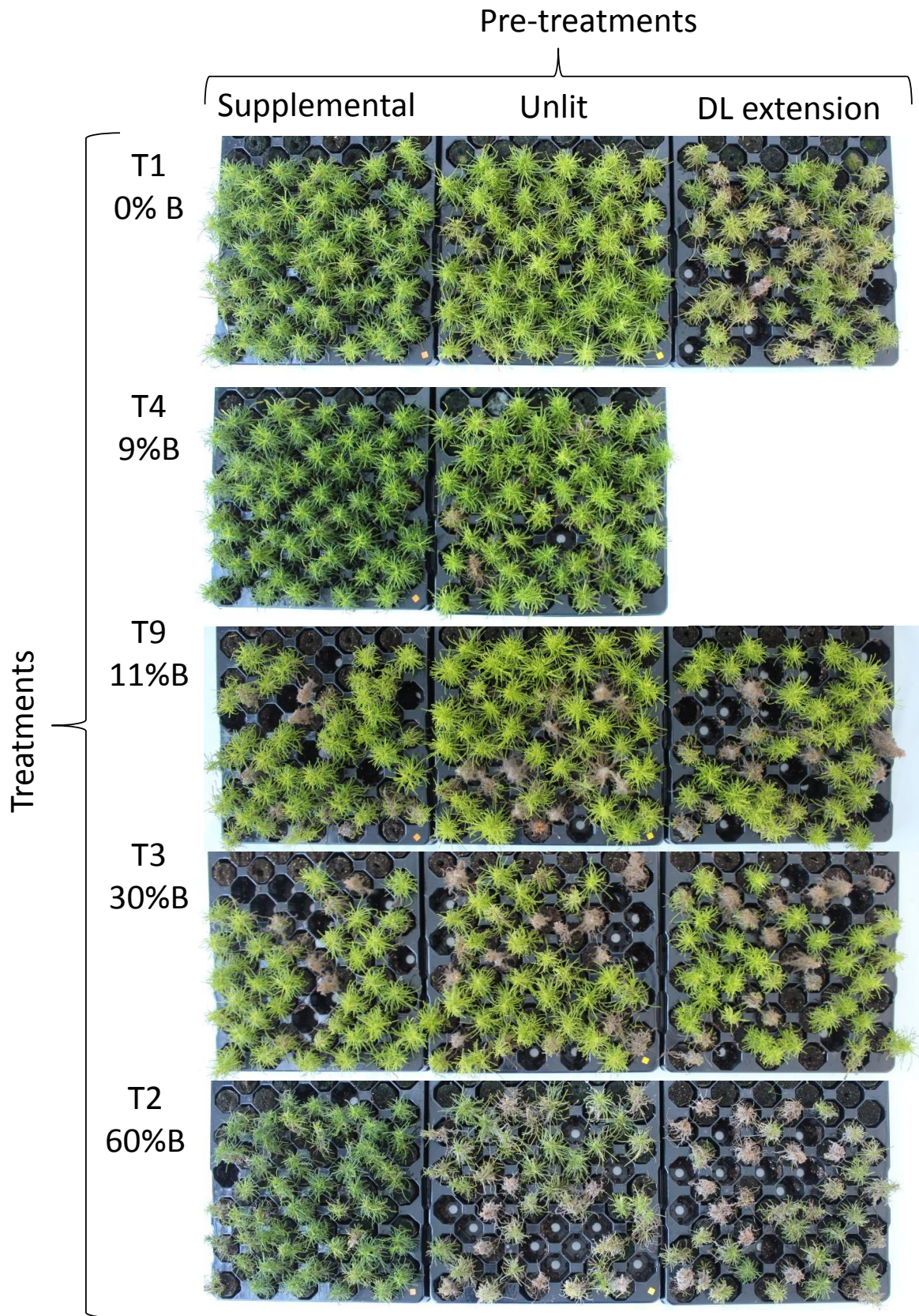


Figure 47. Influence of pre-excision treatments and blue percentage of light-treatments on the appearance of Santolina cuttings after 27 days (18th February 2016) in the LED4CROPS facility.

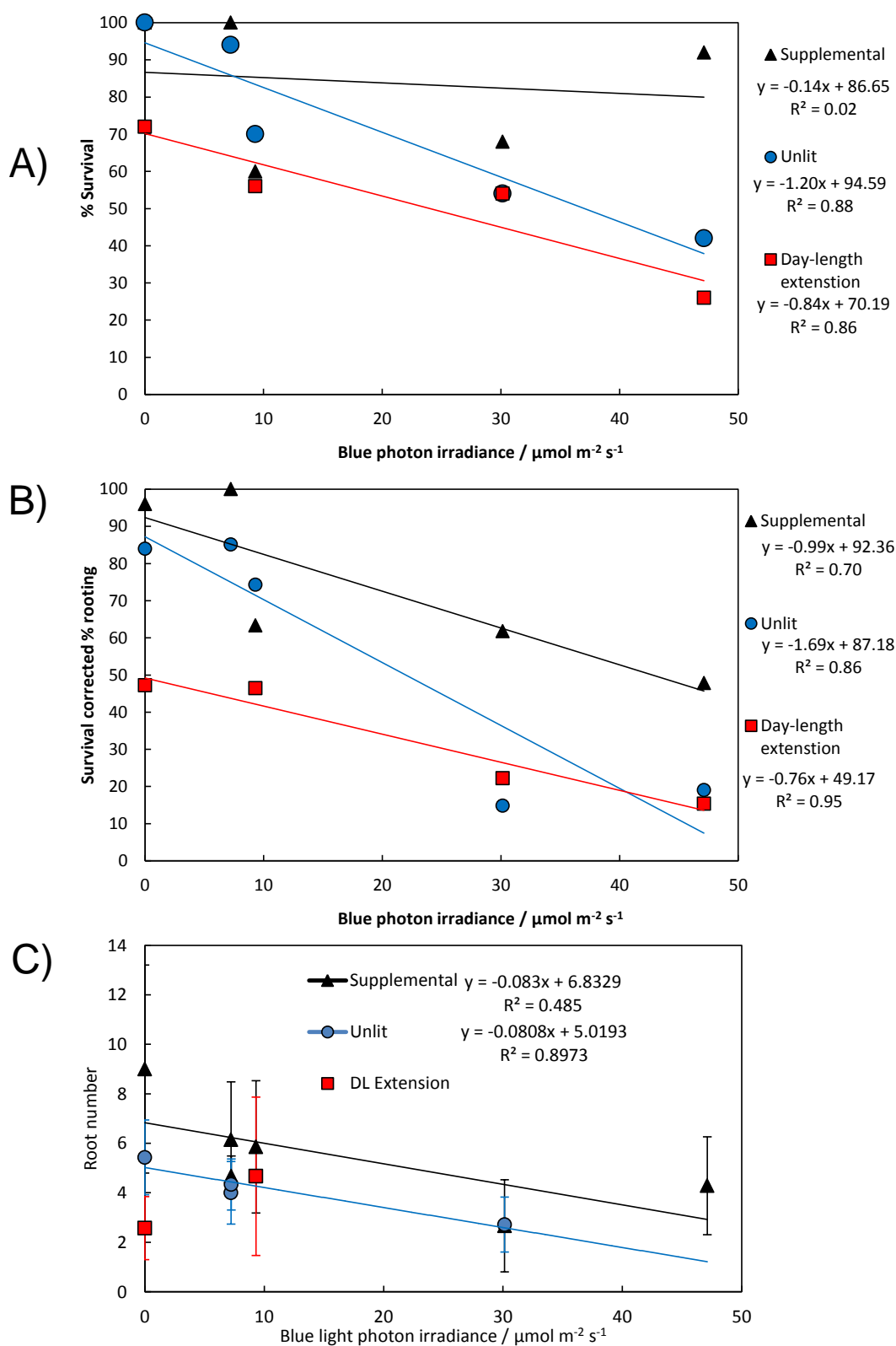


Figure 48. The influence of blue light intensity on **A)** the percentage survival, **B)** the survival corrected rooting percentage and **C)** the number of roots produced by the *Santolina* cuttings from the three pre-excision light treatments (supplemental lighting - $51 \mu\text{mol m}^{-2} \text{s}^{-1}$, Unlit and day-length extension lighting - $5 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Influence of light intensity on Santolina cutting rooting

As described above blue light intensity reduces cutting survival by increased dehydration and potentially via other photobiological mechanisms. While it is possible to alter the light spectrum to reduce blue light intensity it is also possible to lower the blue light intensity by lowering the total light intensity of a red:blue mix. In this experiment we propagated the Santolina cuttings at two lower light intensities, 33 and 68 $\mu\text{mol m}^{-2} \text{s}^{-1}$, of a red blue mix containing 11% blue light. The plants in both of the lower light treatments remained healthy and showed no signs of stress even after 27 days (Figure 49). Cutting survival was 100% in the 33 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 98% in the 68 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment. Survival corrected rooting was 100% in the 33 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 98% in the 68 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment. Number of roots produced was higher in the 68 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment (5.9 roots) than in the 33 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment (4.0 roots) but root lengths were similar between treatments.

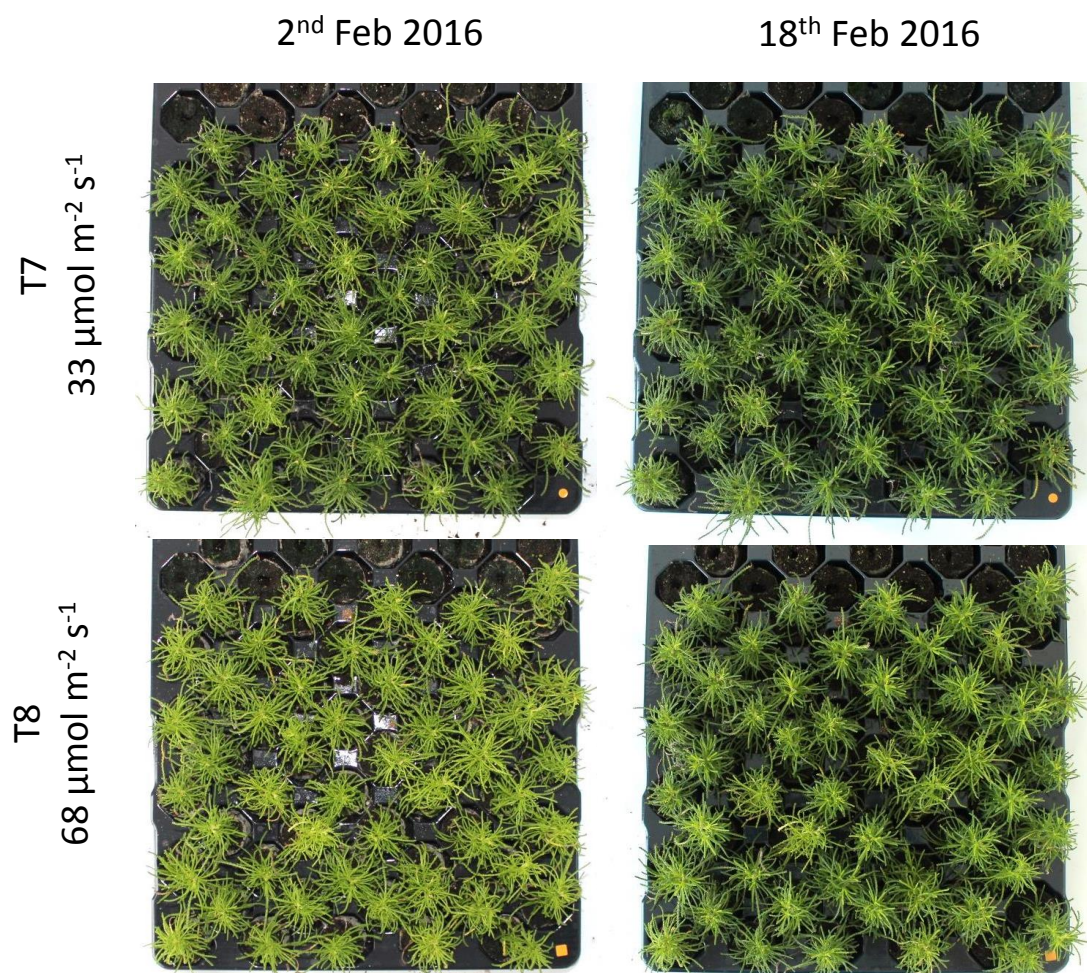


Figure 49. Influence of light intensity on the appearance of Santolina cuttings after 11 days (2nd February 2016) and 27 days (18th February 2016) in the LED4CROPS facility.

Influence of white light on Santolina cutting rooting

As described above light treatments with low blue percentages provide the best results for rooting cuttings. Under red:blue treatments, however, it can be difficult to identify problems such as disease or pest infestations on plants. The white light treatments used in this experiment contained a low blue light percentage (9%, blue) but sufficient green light (18% green) for the plants to appear 'normal', greatly aiding plant assessment. Under this treatment plants remained healthy looking throughout the trial (Figure 50) and cutting survival was 96% and the survival corrected rooting was 92%.

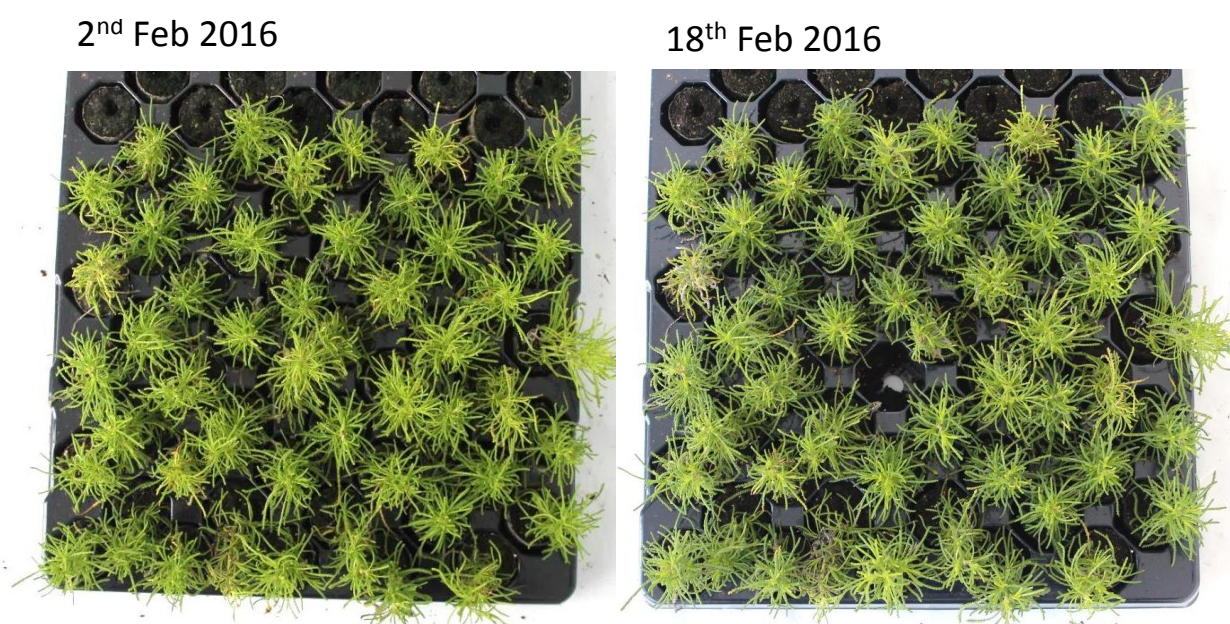


Figure 50. Influence of white light on the appearance of Santolina cuttings after 11 (2nd February 2016) and 27 days (18th February 2016) in the LED4CROPS facility.

Influence of far-red light on Santolina cutting rooting

In the previous year's rooting trial far-red was observed to have a negative impact on cutting survival and rooting. In this trial we compared survival and rooting of the Santolina cuttings under two light treatments with a 9% blue: 91% red light mixture, one with an additional 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of far-red light and one with no far-red. The cuttings propagated under the far-red light treatment showed signs of deterioration after the first 11 days (Figure 51). Cuttings were paler and had some senescing leaves while those under the no far-red treatments remained fresh and healthy. After 27 days exposure to the different treatments the plants under the far-red treatment had deteriorated further while those under the no far-red treatment remained healthy (Figure 52). The supplemental pre-excision treatment cuttings

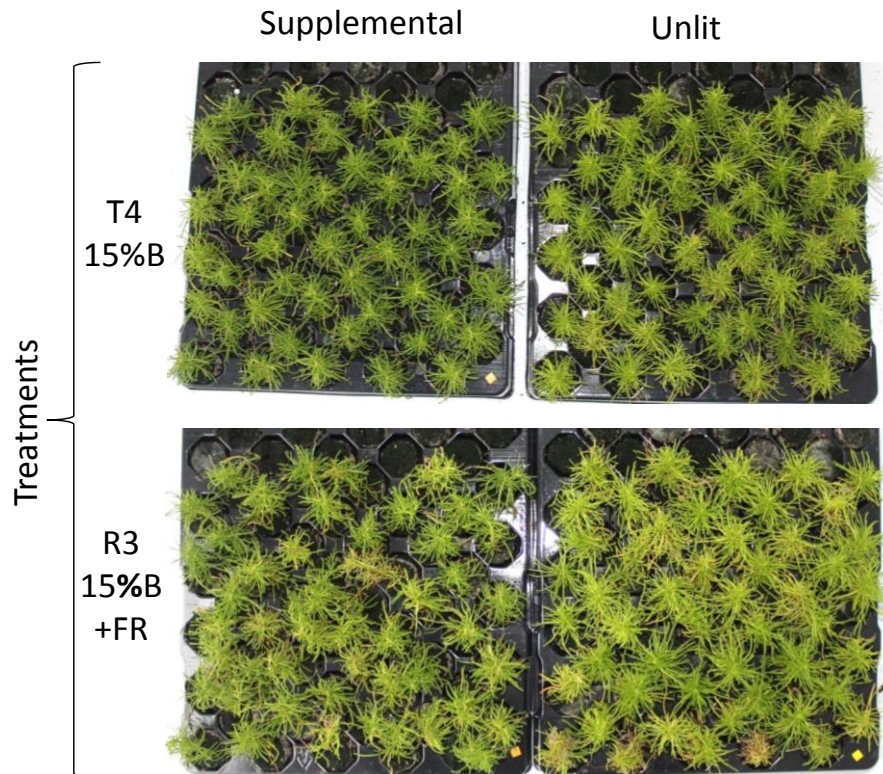


Figure 51. Influence of pre-treatment and far-red light treatment on the appearance of Santolina cuttings after 11 days (2nd February 2016) in the LED4CROPS facility.

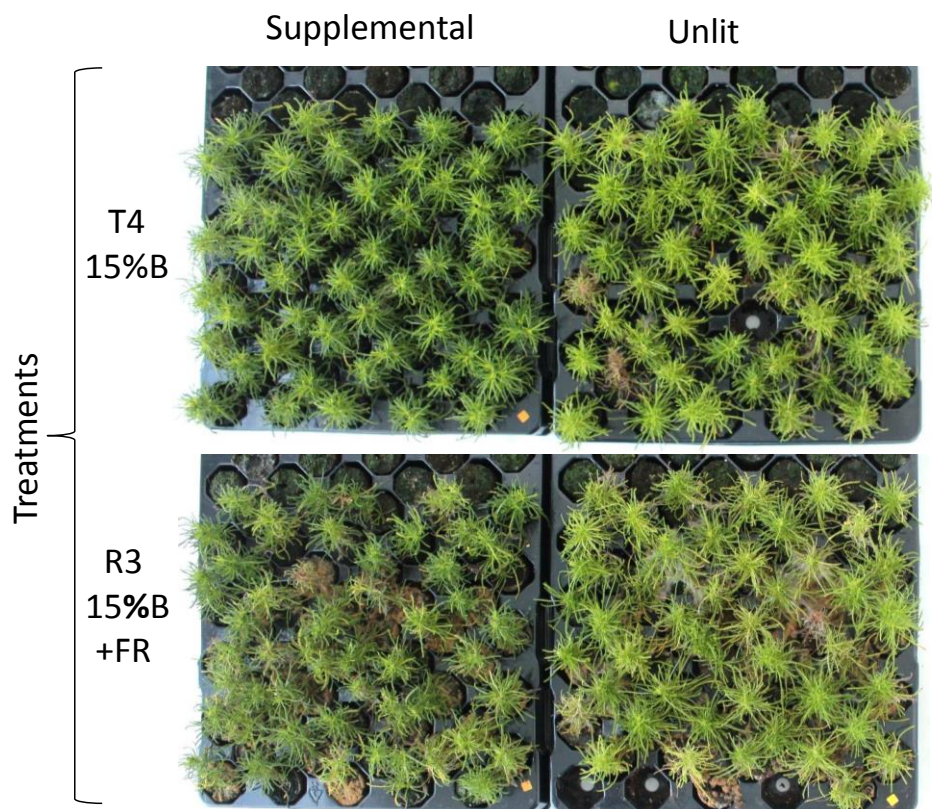


Figure 52. Influence of pre-treatment and far-red light treatment on the appearance of Santolina cuttings after 27 days (18th February 2016) in the LED4CROPS facility.

had a 100% survival and rooting when exposed to the no far-red treatment (Figure 53). When exposed to the far-red treatment, survival remained high (98%) but survival corrected rooting dropped to 59%. The unlit pre-excision treatment cuttings had a 94% survival and 85% rooting when exposed to the no far-red treatment (Figure 53). Interestingly when exposed to the far-red treatment the survival of the unlit pre-excision treatment cuttings decreased to 86% but survival corrected rooting was similar to the no far-red treatment (84%). This suggests some interaction between the plants responses to pre-excision and post-excision light treatments.

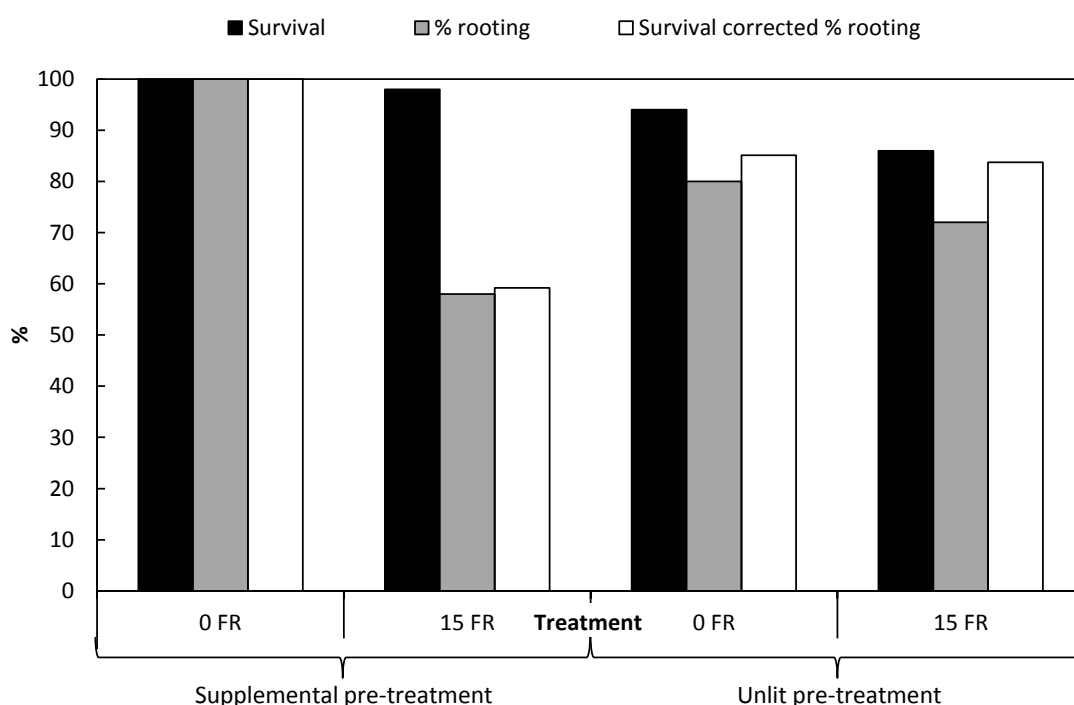


Figure 53. Influence of pre-excision light treatment (supplemental light of $54\mu\text{mol m}^{-2} \text{s}^{-1}$ versus an unlit control) and post excision far-red light treatment on Santolina cuttings percentage survival, percentage rooting and survival corrected rooting percentage.

Clematis ‘The President’

The influence of red:blue light spectra on strike rates of Clematis cuttings

The Clematis tips cuttings remained a healthy green colour in all the treatments, though the cuttings from the 100% red treatments were a slightly paler shade of green (Figure 54). The shoots of the tip cuttings grew during the trial especially those in the 100% red and white light treatments. Higher percentages of blue light reduced the growth of the shoots. No obvious correlation between tip growth and rooting was observed. The leaves of the

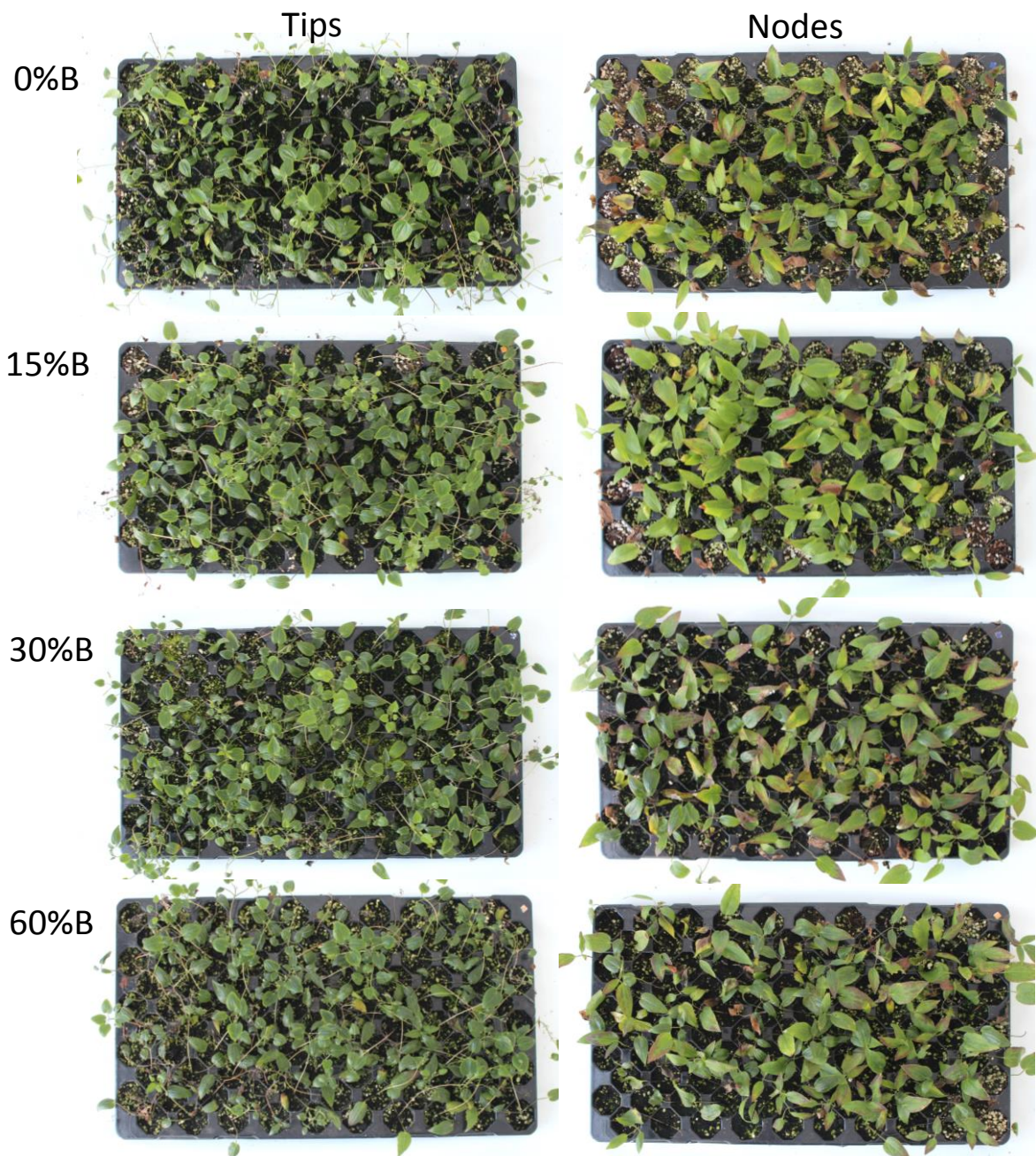


Figure 54. Photographs of the Clematis tip and nodal cuttings grown in the different red blue light treatments. Photographs taken after 5 weeks of exposure to the treatments.

nodal cuttings showed more signs of stress, either yellowing (0 and 15% blue treatments) or purpling (30% and 60% blue treatments), than the tip cuttings. Only one or two of the nodal cuttings showed signs of new shoot growth during the trial.

Survival of both tips and nodes decreased as blue light percentage increased (Figure 55), presumably due to cutting dehydration driven by stomatal opening. Rooting percentage was observed to decrease as blue light percentage increased (Figure 55C). Tip cuttings had on average 13% higher strike rates than nodal cuttings. Under the 100% red light treatment 92% of the tip cuttings rooted while only 73% of the nodal cuttings rooted. Under the 60% blue light treatment rooting decreased to 32 and 47% for the nodal and tip cutting respectively.

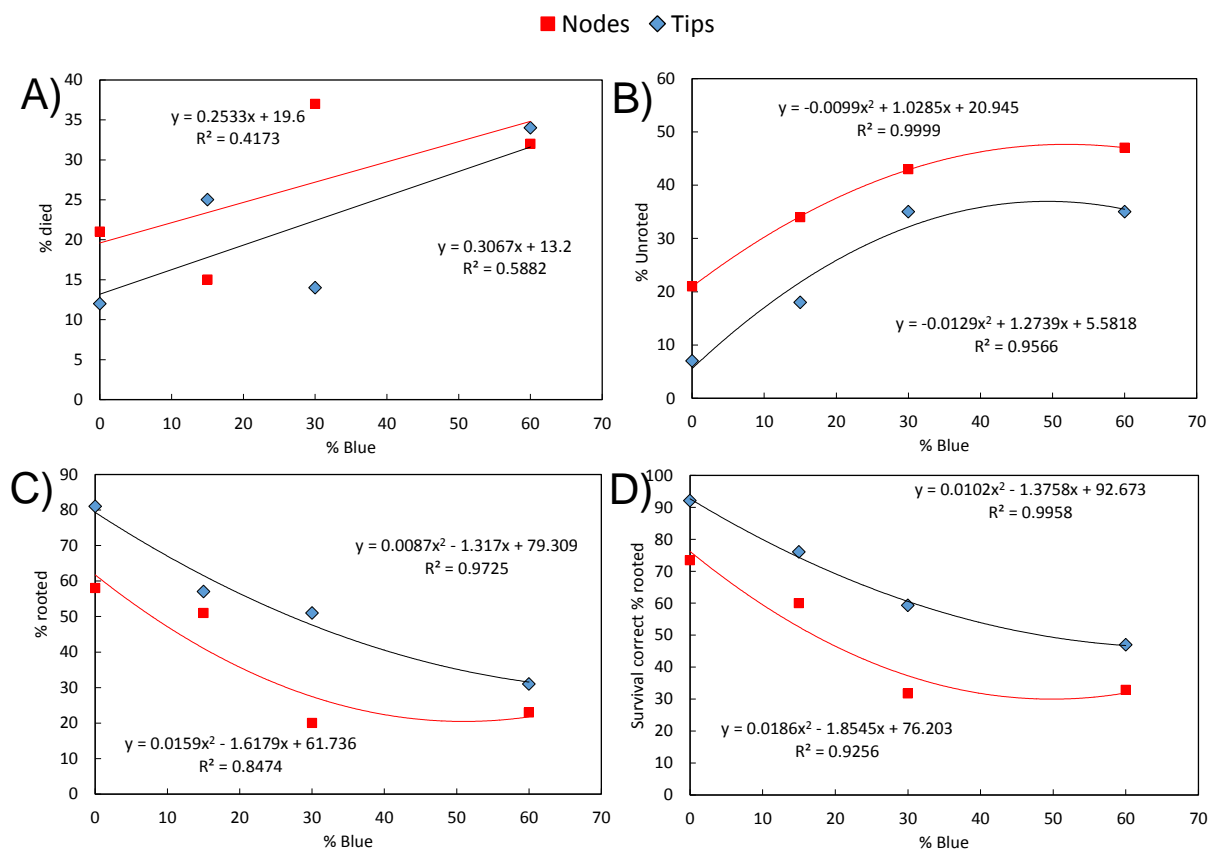


Figure 55. The influence of blue light percentage on the strike rates of Clematis nodal and tip cuttings after five weeks. **A)** Number of cuttings that died, **B)** percentage of cuttings that did not root, **C)** percentage rooting and **D)** the survival corrected percentage rooting.

The influence of far-red light on *Clematis nodal* cutting strike rates

Far-red light was found to have a negative influence on *Clematis* cuttings. The numbers of cuttings that died was greater under treatments with far-red light. Survival correct rooting was found to decrease as far-red intensity increased (Figure 56). The negative impact of far-red light on rooting was also observed in the number of roots produced by the cuttings that successfully rooted, with root number decreasing as far-red increased (Figure 56B).

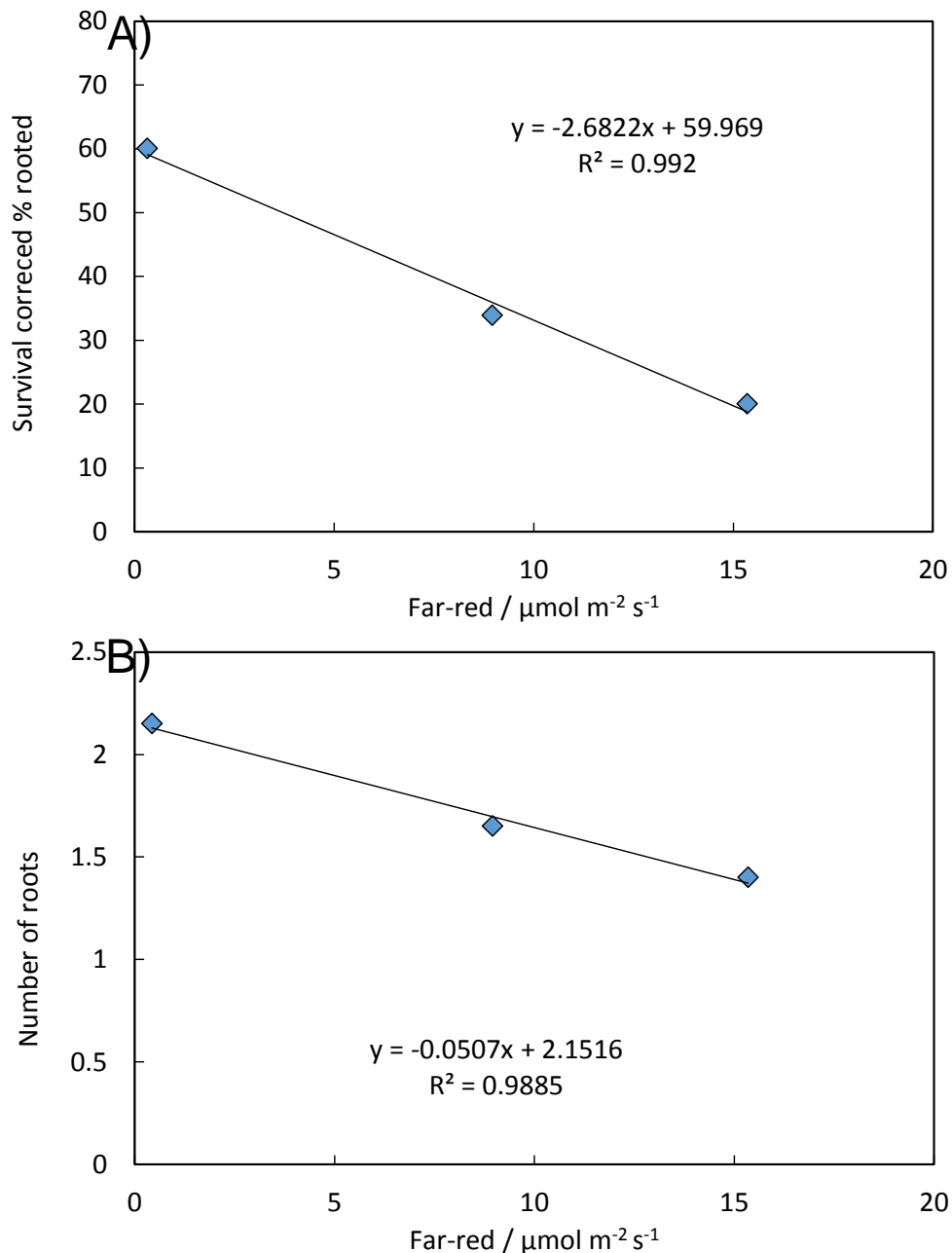


Figure 56. The influence of far-red light intensity on **A)** survival correct rooting and **B)** number roots produced by *Clematis* 'The President' nodal cuttings after 5 weeks exposure to the different light treatments.

Iberis 'Absolutely Amethyst'

The influence of red:blue light spectra on strike rates of Iberis cuttings

Prior to the start of the rooting experiment the appearance of the Iberis cuttings from the two pre-excision light treatments (unlit and supplemental lighting) was similar. After 4 weeks exposure to the different light treatments the cuttings showed few signs of stress even in the 60% blue light treatments (Figure 57). After 4 weeks there were clear signs of shoot growth of the cuttings from some of the light treatments (new growth had a paler green appearance see the photographs in Figure 57). Tip growth was observed to be greatest under the 100% red light treatments and decreased as blue percentage increased. Pre-excision light treatments also influenced shoot growth with more growth occurring, especially in the 100% red light treatment, in the supplemental pre-excision treatment group.

Very few Iberis cuttings died during the four week trial even in the 60% blue light treatment (where only 8% of cuttings died). 100% of cuttings rooted in the 100% red light treatment. Rooting percentage decreased as blue percentage increased with as few as 63% of cuttings rooting in the 60% blue treatment. No difference in rooting percentages were observed between the two pre-excision light treatments. However, the differences in shoot growth between the treatments indicated that the cuttings from the supplemental pre-excision treatment rooted more rapidly than those from the unlit treatment possibly because these cutting had greater stored resources.

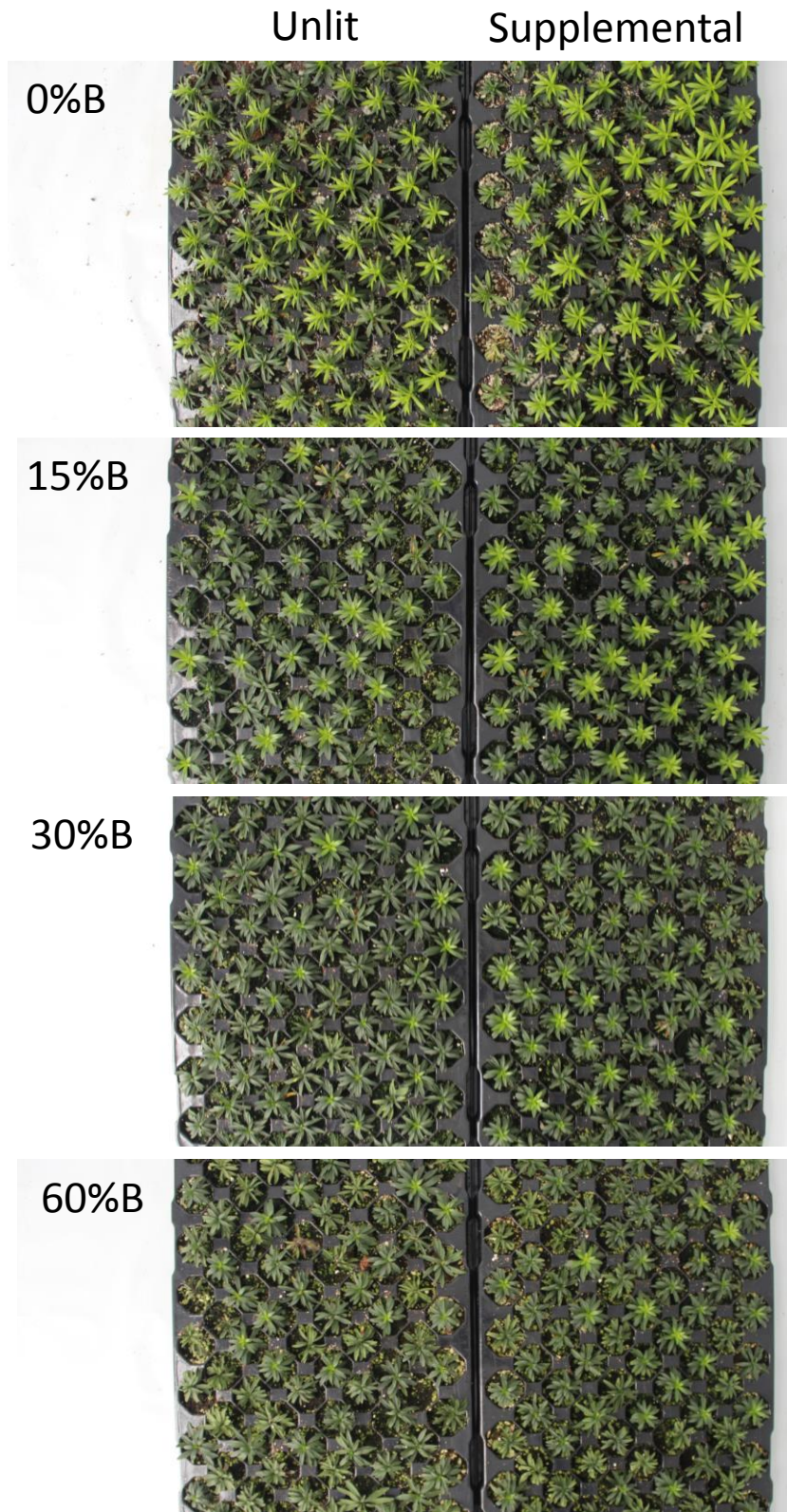


Figure 57. Photographs of Iberis ‘Absolutely Amethyst’ cuttings grown under different light treatments for 4 weeks. Mother stock plants were grown with (Supplemental) and without (Unlit) supplemental LED lighting through the winter months.

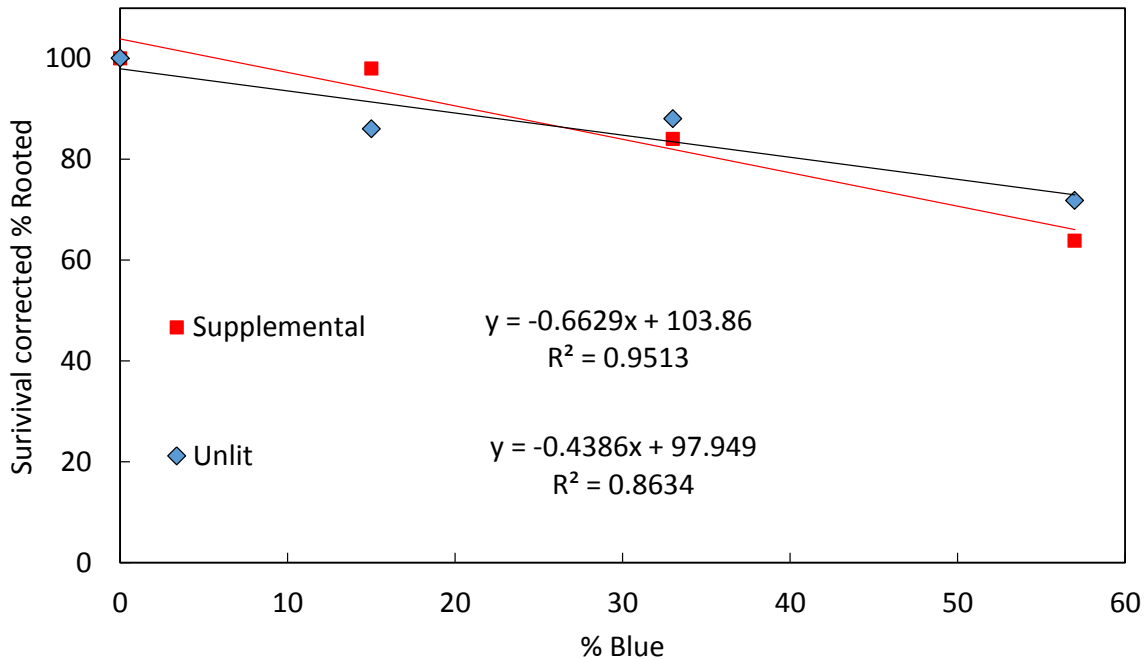


Figure 58. The influence of blue light percentage on the strike rate of Iberis ‘Absolutely Amethyst’ cuttings. Mother stock plants were grown with and without supplemental LED lighting through the winter months.

Discussion WP 2.3. - Improving HNS Propagation

We suggest there are four key factors influencing cutting survival and rooting.

1) Post excision dehydration

Post excision dehydration occurs because the stomata of the cuttings don't close due to the loss of root:shoot signalling. Dehydration is of greatest concern during the first week of propagation. Dehydration was the most likely cause of cutting death observed early in our experiments and the large loss of leaves observed in the Eleagnus cuttings examined during the year one trials. Post excision light treatments have a pronounced effect on cutting dehydration. Blue light provides an important signal for opening stomata and removing or reducing the amount of blue light in the spectrum can reduce stomatal opening and dehydration resulting in better cutting survival.

2) Dehydration influenced changes in hormone status

Dehydration of cuttings causes changes in the hormonal status of the cuttings. These hormonal changes act to close stomata and this is why cuttings become less susceptible to dehydration over time. Because the light environment influences the dehydration process via stomatal opening light indirectly influences this process. Rapid dehydration can cause leaf excision as observed in the Eleagnus cuttings trial in the year one trials. Slower rates of dehydration may allow the hormone status to change more gradually allowing stomatal

closure prior to 'terminal' dehydration. How well this process occurs may impact how much stress cuttings undergo during the rooting process.

3) Direct light induced changes in hormone status

The light environment a cutting is exposed to has a direct influence on both hormone production and transport. These factors are expected to have a major influence on the survival corrected rooting measurements presented in this report. Hormonal changes occurring in response to 2 and 3 may interact to influence rooting.

4) Internal cutting resources

The resources available to a cutting will have a major influence on its ability to grow new roots. Cutting photosynthesis may contribute a small amount of resources but it is unlikely to have a large impact on cutting growth until roots have developed and the stomata reopen. Large differences in resource availability are expected between species based on where in the plant resources are stored and the quantity of resources stored. The conditions mother stock plants are exposed to will have a major impact on the quality of cuttings and the quantity of starches and sugars stored in those cuttings. All of these factors will contribute to overall cutting strike rates but a greater understanding of the different factors may help optimise the conditions and light environment cuttings are exposed too during propagation.

In these experiments post excision light treatments had large influence on cutting strike rates of all three species examined. As observed during the cutting trial from year 1, increasing blue light intensity was associated with increased cutting dehydration probably due to the increased stomatal activity under these treatments. This increased stress had its greatest impact on cutting survival on weaker cutting material (Santolina cuttings from day length extension pre-excision treatments). The survival corrected rooting data suggested there was a considerable influence of light quality on the root induction of cuttings. Rooting was observed to decrease as blue intensity increased. Rooting percentages were high in all treatments with low blue intensities including low blue percentage red:blue mixtures, low intensity red:blue and white light treatments. It is possible that red light is important in production and transport of the auxin that is required for rooting.

The inclusion of far-red in the post excision light treatments was observed to reduce cutting survival and rooting efficiency of Santolina and Clematis cuttings. We have some evidence that far-red light may cause an increase in stomatal conductance possibly due to changes in hormonal status. Far-red light is also expected to counteract any red light influences.

Further work is required to determine which hormones are influenced by the different regions of the light spectrum.

Conclusions WP 2.3 - Improving HNS Propagation

The post excision light quality provided to cuttings has a large impact on both their survival and ability to root. Rooting and survival were greatest under 100% red light and lowest under 60% blue light. It is thought that blue light induced stomatal opening caused cutting dehydration, especially during the first week after sticking, and this reduces survival and rooting. There also appears to be a spectral influence on cutting hormonal status which influences the speed and extent of rooting. While 100% red light provided the best rooting any shoot growth that occurred under this light treatment was etiolated. Plants would need to be transferred to a spectrum including some blue light, as soon as they have rooted, to maintain plant morphology and promote vigour. Far-red light was found to reduce rooting. This suggests that the far-red light was reducing some of the benefits to rooting provided by red light.

Light treatments provided to the mother stock plants were also found to have a significant influence on rooting and survival. Mother stock plants provided with supplemental lighting produced healthier cuttings with better survival than those from unlit mother stock plants. Mother stock plants lit with night break lighting produce weaker cuttings than those provided by unlit mother stock plants. Providing the correct lighting before and after cuttings are collected will allow maximum success.

WP 3 - Light quality and its influence on pests

Introduction

Relatively little is known about the potential positive or negative effects that LED-based plant production within enclosed systems (i.e. independent of sunlight) may have upon pest insects and biological control organisms. Nevertheless, it is likely that in systems such as those used in the current project, where UV/green light is absent, effects on visually-based insect orientation, dispersal and host location could be expected, with many invertebrates having evolved tri-chromatic vision most active at blue, green and UV wavelengths. Indirect effects, mediated through changes to plant chemistry, could also be expected, particularly given the pivotal role that UV (B) is known to play in modulating and/or upregulating plant defence chemistry (along with R:FR ratios). The degree to which different light regimes effect different pest and beneficial insects is therefore likely to be dependent on the degree to which the target organism relies upon vision/chemistry in host (plant or pest) location, as well as the degree to which the plant responds to varying light treatment, innately or post-pest infestation. To further complicate matters, under certain light regimes, physical plant responses, as well as chemical responses, could potentially render some plants more or less susceptible to pest attack (e.g. leaf thickening/curling).

The above in mind, plant-pest-beneficial insect responses to LED-based production are likely to be highly complex. Whilst it would not be possible to fully understand such responses within the current study, a series of experiments were nevertheless planned with the aim of providing preliminary data to demonstrate responses of pest and beneficial invertebrates to cropping systems based on varying ratios of red and blue light, comparing to white light as a control. Both aphids and spider mite were selected as target pest species (based on general pest significance and varying feeding strategies), with parasitoid wasps and predatory mites selected as pest natural enemies for investigation (based on varying life histories and visual acuities). Target crops were selected following discussions with industry representatives, though in some cases had to be substituted for alternatives to ensure pest development in control treatments on more suitable hosts.

Pest responses to sticky traps under LED-based production were reported in year 1. This report contains a series of trials aimed at understanding pest responses and biocontrol responses to LED lighting. The experiments reported are arranged in to three work packages.

WP 3.3a Determining the effects of plant physiology on aphid development

WP 3.3b Determining the effects of plant physiology on spider mite development

WP 3.4a Evaluation of bio-controls for aphid

Within these work packages each trial is reported with specific methods due to the different approaches taken with different crops and pests. Future studies planned for 2016/2017 will investigate predatory mite efficacy against spider mite, and 'long range' parasitoid host location.

WP 3.3a Determining the effects of plant physiology on Aphid development

Peach potato aphid individual responses on Lettuce and Verbena

Methods

Fifty plants in individual pots were grown under five different LED light treatments (Table 10), such that there were ten plants per treatment: (1) white light control; (2) 100% blue light; (3) 60% blue : 40% red light; (4) 30% blue : 70% red light; (5) 100% red light.

Table 10. The specification of the four light treatments selected for experiments under Work Package 3.

Light treatment	100 % B	66 % B	33% B	0% B	White Control
Location	R1A or R1C	R2C or R1C	R4C or R1B	R3B	P7C
PAR / $\mu\text{mol m}^{-2} \text{s}^{-1}$	145	200	200	200	200
DLI / mol m^{-2}	8.4	11.5	11.5	11.5	11.5
Blue photon irradiance	145	132	66	0	12
Red photon irradiance	0	68	134	200	173
Green photon irradiance	0	0	0	0	15
% blue	100	66	33	0	6

Two sets of three trials were conducted for each of two plant types: Lettuce (*Lactuca sativa* var. *capitata* 'Amica' RZ) and Verbena (*Verbena* sp.), to allow for randomisation of light module bench location and staggering through time. A total of thirty plants per light treatment were therefore used across the three trials for each of the plant types.

Performance indicators

One adult peach potato aphid from a stock culture (maintained on the same type of host plant as the experimental plants at 20°C under a 16L:8D hr photocycle) was caged on each of the plants with a clip cage at the start of each experimental trial. Each cage was left undisturbed for 24 hrs and was then inspected. Where nymphs had been deposited, one was selected at random as the experimental aphid, whereas any remaining nymphs and the adult were removed. The experimental aphid was then left to develop undisturbed in the cage.

Seven-day fecundity

The number of nymphs deposited by each experimental aphid from days 1 to 7 of the aphid's reproductive period was counted, and the sum total gave the seven-day fecundity. Where an experimental aphid died before the elapsing of all 7 days, the number of nymphs deposited on remaining days was recorded as zero, rather than only summing over the shorter period.

Pre-reproductive period (D)

The pre-reproductive period was defined as the number of days taken after birth for the experimental aphid to start depositing its own nymphs.

Mortality

Pre-reproductive mortality was recorded, and the percentage proportion of experimental aphids dying before the deposition of their first nymphs established. A record of the number of reproductive days was also noted for experimental aphids, to provide an indication of proportions not surviving for a number of days equal to D or to the minimum seven days required for seven-day fecundity calculation.

Intrinsic rate of increase (r_m)

The number of nymphs produced by each experimental aphid for the same number of pre-reproductive days of the aphid. The r_m was calculated using the following formula (Wyatt and White, 1977):

$$r_m = 0.74 \left(\ln \frac{F_D}{D} \right)$$

where F_D is the number of nymphs produced over a period of time equal to the pre-reproductive period (D). Where an experimental aphid died before the elapsing of a number of days equal to D , the number of nymphs deposited on remaining days was recorded as zero.

Plant chemical analyses

Plant foliar material was collected and sent for full foliar analysis to NRM, as per their requirements. A minimum of 200g of foliar tissue was supplied, as a mixture of material of all ten plants per light treatment in each trial repetition.

Statistical analysis

All statistical analyses were carried out using the statistical software, R (version 2.15.1, R Development Core Team (2012)).

Aphid performance was assessed using linear mixed effect models, using the 'lme4' package (Bates *et al.*, 2012) as per Bolker *et al.* (2009), and checked for significance using the 'car' package (Fox and Weisberg, 2011). Aphid performance was analysed using light treatment as a fixed effect, and trial as a random effect (pot number was nested into trial). A post-hoc Tukey's HSD test was used to compare between light treatments where significance was observed, using the 'multcomp' package (Hothorn *et al.*, 2008).

In order to assess the effects of LED light regime on pre-reproductive mortality, raw data were transformed to percentage mortality under each treatment. These data were then analysed using ANOVA following arcsin square-root transformation (to account for the data being bounded and to meet test assumptions for normality of errors). Model simplification was carried out as per Crawley (2007).

The effects of LED light regime on plant chemistry, and aphid performance response to any differences, were assessed using one-way and two-way ANOVA, respectively, as per Crawley (2007).

Results

Aphid Reproductive Performance

Number of pre-reproductive days (D)

The number of pre-reproductive days (Table 11) was found to be affected by light regime ($F_4 = 11.16$, $P < 0.05$); where aphids reared under 60% blue light were found to have a greater number of pre-reproductive days than under the white control ($P < 0.05$), 100% blue ($P < 0.05$) and 30% blue ($P < 0.05$) light treatments.

Seven-day fecundity

Seven-day fecundity (Table 12) was not affected by light regime ($F_4 = 4.99$, n.s.).

Intrinsic rate of increase (r_m)

Intrinsic rate of increase (Table 13) was not affected by light regime ($F_4 = 7.83$, n.s.).

Table 11. Number of pre-reproductive days (mean \pm SE) under the different light regimes, in the three replicate trials.

	White light control	100% blue light	100% red light	30% blue light	60% blue light
N. pre-reproductive days	7.21 \pm 0.41	7.45 \pm 0.32	7.00 \pm 0.45	8.09 \pm 0.44	9.67 \pm 1.76
Trial 1	8.25 \pm 1.11	8.43 \pm 0.43	10.00 \pm NA	7.50 \pm 0.50	NA
Trial 2	7.00 \pm NA	6.60 \pm 0.60	5.00 \pm NA	9.5 \pm 2.50	13.0 \pm NA
Trial 3	6.78 \pm 0.36	7.13 \pm 0.48	6.88 \pm 0.30	7.86 \pm 0.26	8.00 \pm 1.00

'NA' indicates that there were not enough successful replicates for value calculation.

Table 12. Seven-day aphid fecundity (mean \pm SE) under the different light regimes, in the three replicate trials.

	White light control	100% blue light	100% red light	30% blue light	60% blue light
7-day fecundity	5.29 \pm 0.92	6.05 \pm 1.04	3.60 \pm 0.67	3.27 \pm 0.49	2.00 \pm 0.58
Trial 1	9.25 \pm 1.55	9.57 \pm 2.23	9.00 \pm NA	5.50 \pm 0.50	NA
Trial 2	1.00 \pm NA	5.20 \pm 1.56	2.00 \pm NA	2.50 \pm 1.50	1.00 \pm NA
Trial 3	4.00 \pm 0.62	3.50 \pm 0.42	3.13 \pm 0.35	2.86 \pm 0.46	2.50 \pm 0.50

'NA' indicates that there were not enough successful replicates for value calculation.

Table 13. Intrinsic rate of aphid increase (r_m) (mean \pm SE) under the different light regimes, in the three replicate trials.

	White light control	100% blue light	100% red light	30% blue light	60% blue light
R_m	-0.42 \pm 0.16	-0.32 \pm 0.14	-0.56 \pm 0.08	-0.72 \pm 0.14	-1.21 \pm 0.34
Trial 1	0.09 \pm 0.12	-0.05 \pm 0.29	0 \pm NA	-0.23 \pm 0.02	NA
Trial 2	-1.44 \pm NA	-0.33 \pm 0.32	-0.68 \pm NA	0.98 \pm 0.46	-1.90 \pm NA
Trial 3	-0.53 \pm 0.18	-0.54 \pm 0.10	-0.62 \pm 0.07	-0.78 \pm 0.16	-0.87 \pm 0.06

'NA' indicates that there were not enough successful replicates for value calculation.

Aphid pre-reproductive mortality

Aphid pre-reproductive percentage mortality (Table 14) was found to differ by treatment ($F_{4,8} = 5.14$, $P < 0.05$) and by trial ($F_{2,8} = 11.70$, $P < 0.01$); where mortality was increased under 60% blue light ($t = 3.07$, $P < 0.05$) when compared with a white light control, and where mortality was lower in trial 3 than that in the other trials ($t = -3.98$, $P < 0.01$). An interaction between treatment and trial was not supported.

A post-hoc Tukey's HSD test also indicated a pairwise difference between mortality under 60% blue light and 100% blue light ($P < 0.05$), with mortality under the former being greater than that of the latter.

Table 14. Aphid mortality (mean \pm SE) under different light regimes in the three replicate trials (mean).

	White light control	100% blue light	100% red light	30% blue light	60% blue light
Percentage mortality	53.3 \pm 23.3	33.3 \pm 8.82	66.7 \pm 23.3	63.3 \pm 16.7	90.0 \pm 5.8
Trial 1	60	30	90	80	100
Trial 2	90	50	90	80	90
Trial 3	10	20	20	30	80

Melon aphid population responses on Verbena

Methods

A small segment of Verbena leaf with approximately 20 melon aphids of mixed age was removed from cultures maintained at STC for use in the experiment. An experimental plant was then artificially infested by placing this leaf section on a leaf midway up the stem of a 10 week old Verbena plant. Experimental plants had been trimmed down to approximately 15cm in height and bread-bagged to prevent aphid escape into the LED unit at STC. After 10 days under white illumination in the insect culture room, the leaf section was removed, and the number of aphids assessed. Thirty plants with 7-40 live aphids upon them were then assigned to treatment in the LED unit at STC (see Table 10) by random reciprocal ordering, after which they were placed in the LED unit for 10 days. The plants were then destructively harvested and the aphid population assessed. Six simultaneous repeats were performed to allow statistical analysis of the data (using ANOVA and post-hoc Tukey Tests in R).

Results

Under the blue/red light mix, aphid fecundity was impaired relative to under the white light control (Figure 59). Population growth was also notably lower under the 100% red treatment as compared to when blue light was provided, but the amount of blue light made no apparent difference.

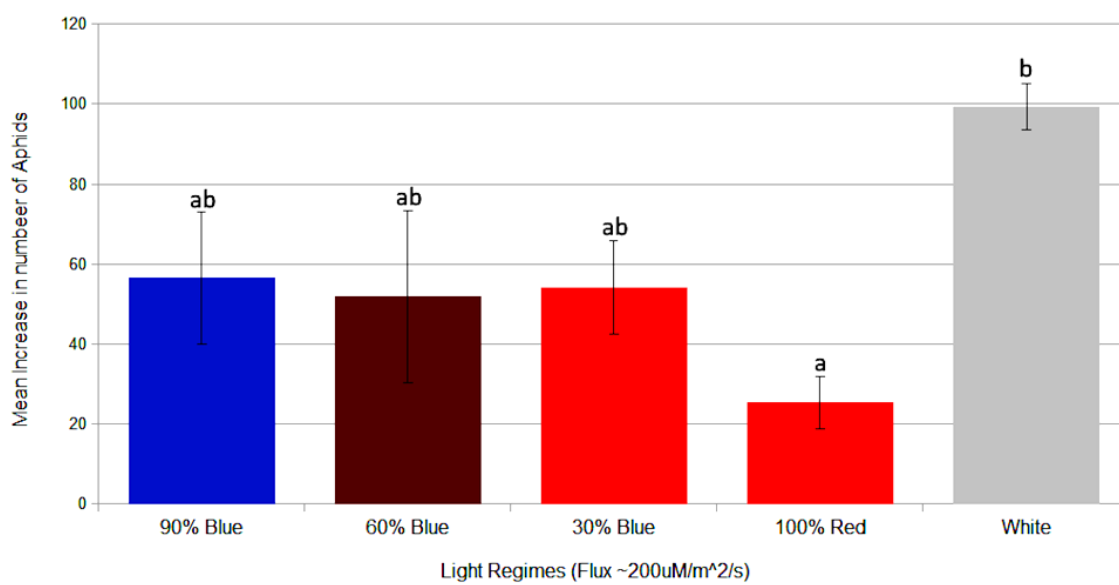


Figure 59. Average net increase in number of aphids counted on plants after 10 days under different ratios of red and blue LED illumination at $200\mu\text{mol m}^{-2} \text{s}^{-1}$, and in a white light control (sunlight mimicking, but UV negative, LED mix). Error bars are standard error at $n=6$, and letters indicate significance groupings according to a TukeyHSD test ($p<0.05$).

WP 3.3b Determining the effects of plant physiology on spider mite development

Two-spotted spider mite responses on Cucumber

Methods

Individual Cucumber plants (*Cucumis sativus* Var. Carmen) were sown into individual pots on 18/01/2016 (using Levington M2 potting and bedding compost) and placed under the specified light treatments (see Table 10). Once all plants obtained a minimum of two true leaves, vials containing 20 adult spider mite were added at the base of each plant and enclosed upon the plant by fixing a bread bag over the plant to the pot base. These were split into two runs on different days (05/02/2016 and 08/02/2016) with three plants in each treatment being infested on each day. Infested plants were kept under the light treatments for a further 14 days. At 14 days after first infestation, the plants were removed from light

treatments and individual leaves were studied with the use of a microscope. Adult and juvenile spider mite were counted.

Results

Two-way ANOVA of the data revealed no significant differences between start dates (Adult- $F(1, 20)=1.195$, $P=0.2873$; Juvenile- $F(1, 20)=1.308$, $P=0.2663$; Total- $F(1, 20)=1.485$, $P=0.2372$), hence allowing for the conjoining of these data sets to produce more powerful results.

Analysis using one-way ANOVA and post-hoc Tukey's test did reveal statistical differences between some treatments (ANOVA: Adult- $F=6.444$, $P=0.0010$; Juvenile- $F=2.929$, $P=0.0409$; Total- $F=4.075$, $P=0.0112$), with spider mite population growth being consistently lower under white light and the 30B/70R treatment than the 90B/10R or 100% red treatments (Figure 60).

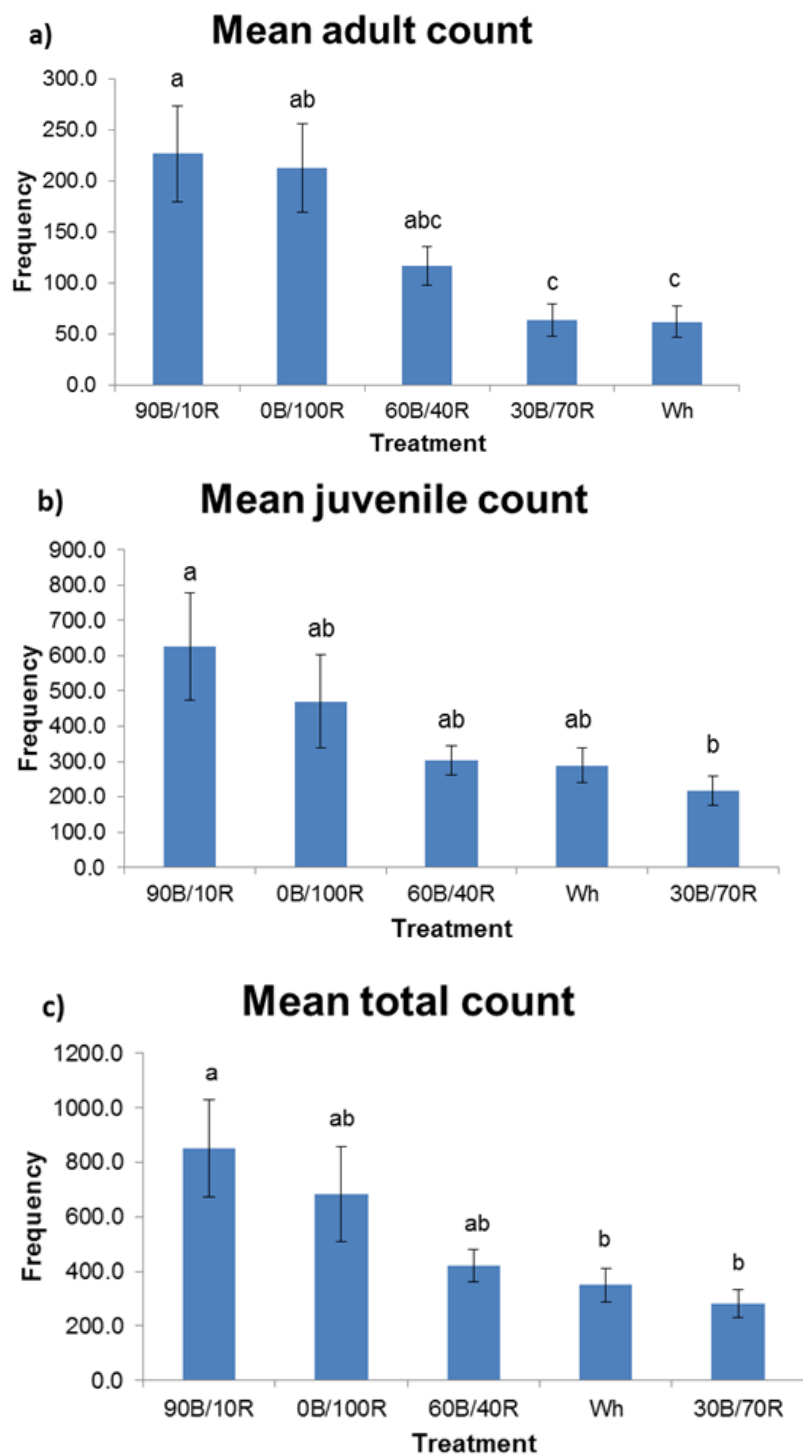


Figure 60. Mean number of spider mites per plant in each treatment. Error bars represent the standard error of the mean and Tukey's test significances. Letters above bars correspond to the results of the Tukey tests, where means not sharing a common letter are significantly different.

WP 3.4a Evaluation of bio-controls for aphid

Parasitism of peach potato aphid on Chinese cabbage

Method

Twenty peach potato aphids were removed from a stock cage of cabbage with a soft brush and placed on leaves of bread-bagged Chinese cabbage plants, grown under standard glasshouse conditions at STC. These were then left for two days (under white light) in the insect growth room at STC to acclimatise. Wasps were then added, and the plants placed under the LED treatments (see Table 14) for 24 hours, the wasps removed, and the plants returned to the insect growth room for 10 days to allow mummies to form. The plants were then destructively harvested, and numbers of aphids and mummies assessed. Mummies were placed in large vented Petri dishes sealed with parafilm for a further 10 days, and the number of emerged wasps was then counted.

Two repeats (Trial 1 & 2) were performed, which due to time constraints had to use different batches of wasps obtained from Koppert a week apart. The batch of wasps used for Trial 2 displayed much shorter lifespans, so much of the initial hatching flush died before use. Therefore, newly hatched wasps had to be used, and a male wasp was added to ensure that females would be mated.

- Trial 1 – two female wasps between 12-36 hours post emergence and post-arrival of the wasp mummies from the supplier. Six repeats were performed simultaneously.
- Trail 2 – two female and one male wasp <24 hours post-emergence and 3 days post-arrival were used. Seven repeats were performed simultaneously.

Results

A multi-way ANOVA of the pooled results showed the only significant variable to be 'repeat', with the second trial showing greater reproductive success. Therefore, the unpooled data is presented below. Though not statistically significant, in both trials, the 30% blue treatment had the most successful reproduction, larger than the higher blue ratios and the white control. The 100% Red treatment varied most between repeats, appearing the same as the (non-30%) blue treatments in Trial 2, but appearing to perhaps inhibit reproductive success in Trial 1.

Rates of reproduction in *Aphidius matricariae* on *Myzus persicae* & Cabbage under different LED illumination

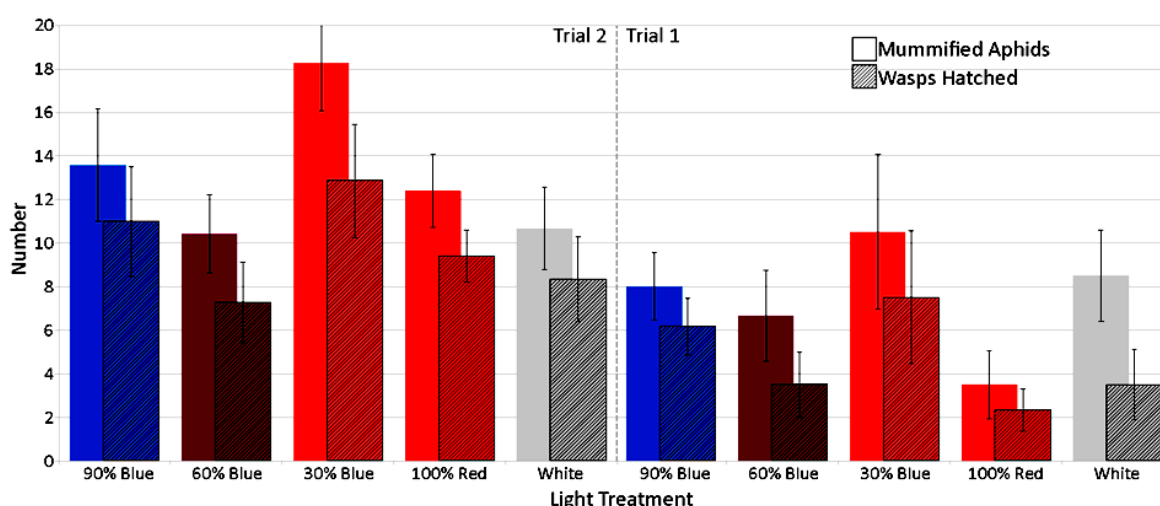


Figure 61 Mean number of mummified aphids (clear bars) 10 days after exposure to two female wasps for 24 hours, and the number of new wasps that had hatched from those mummies after a further 10 days. Error bars are standard error at n=6 (Trial 1) and n=7 (Trial 2).

WP 3.4a Parasitoid wasp activity

Method

Forty wasps (*Aphidius matricariae*; mixed unknown sex, less than 24 hours old) were placed inside insect cages (BugDorm-44545, MegaView Science Co.; with vertical supports cut down by 5cm to fit the cages to the height of the LED benches), with a cotton-wool bud dipped in a solution of 50% table-sugar for sustenance. Sticky traps to monitor wasp flight activity were made from the lid of a Petri dish (~255cm²), coated with a thin layer of insect glue (Raupenleim hell; Schacht) and suspended in the middle of the cage. Wasps were left for 24 hours in four different light treatments (see Table 10), as well as white light and complete darkness controls, all within the LED growth facility at STC. The percentage of wasps caught in sticky traps was then subjected to statistical analysis by ANOVA and Tukey Tests in R.

Results

As expected, wasps flew far less often in constant darkness than in a normal day-night cycle. In the presence of blue or mixed blue/red light, wasps flew fairly consistently, at similar levels to white illumination. There was a slight, but non-significant, decrease in flight activity with decreasing blue illumination. The 30% Blue treatment was the same as the white light control (also containing the ~30% blue wavelengths seen in sunlight), which

supports the reliability of these results. The 100% Red treatment had significantly less flight activity than the other light treatments, but still considerably more than the dark control.

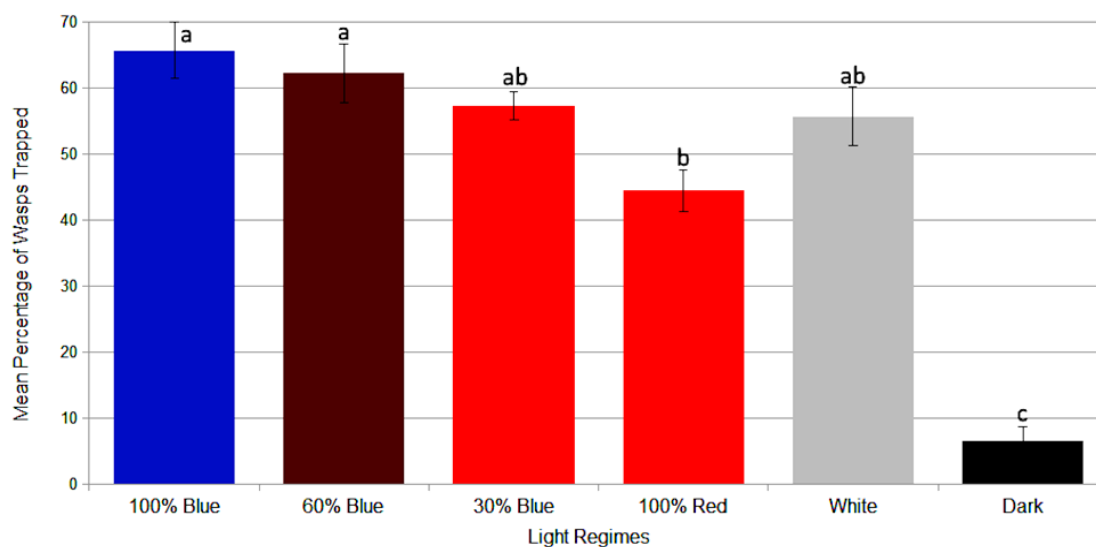


Figure 62. Mean percentage of wasps caught in a colourless sticky trap only accessible by flight during a single 24 hour day/night period under different ratios of red and blue LED illumination, and in dark and white light (sunlight mimicking, but UV negative, LED mix) control conditions. Error bars are standard error at n=8, and letters indicate significance groupings according to a TukeyHSD test ($p < 0.05$).

Discussion

Detailed experiments investigating individual peach potato aphid responses demonstrated that light treatments can have significant effects on the performance of this pest species, most likely mediated via the light responses of the host plants rather than a direct response on the insects. In Lettuce plants peach potato aphid survival and fecundity were significantly lower on plants grown under 60% blue light compared to those under 100% red or 100% blue light. However, when the same strain of aphid was cultured on Verbena plants grown under the same light treatments, the survival and fecundities were unaffected by light treatment. The specific response of the Lettuce plants that caused this drop in insect fecundity remains to be defined, though it was noted that the aphids appeared to struggle to attach and feed on the Lettuce plants grown under the 60% blue light, resulting in the high mortality rate in this treatment. Difficulties in feeding may have been associated with changes in leaf structure or leaf surface chemistry. It is also possible that under this light treatment the Lettuce plants were unpalatable due to the presence of secondary metabolites within the leaf that deterred aphid feeding. It was also noted that the plants under the 60% blue light treatment were the slowest growing Lettuce plants; it is plausible that this may have reduced the sugar concentration of the sap and therefore its acceptability to aphids. Aphid fecundity has previously been shown to be influenced by the nutritional

value of host plants, with higher nitrogen contents resulting in higher fecundity. However, plant tissue analysis indicated that leaf plant nitrogen concentration increased as blue light percentage increased, suggesting that nutritional value of the Lettuce plants was not a major driver of treatment effects, at least in terms of N.

When population responses of a different aphid species (melon aphid) were assessed on Verbena, lowest population success was observed under the 100% red light treatment, potentially demonstrating that effects are pest species specific, as well as plant specific (as 100% red light appeared to have minimal impact on peach potato aphid on Verbena plants). It deserves note, however, that in the experiment with melon aphid plants were initially cultured under natural/white light, making direct comparison between experiments difficult. Nevertheless, a negative response of the aphid population to red light could potentially be explained by the aphid's inability to perceive red light, with a resultant collapse of circadian rhythm and the expected loss of fitness that might entail. A dark control would have confirmed this hypothesis, but could not be performed due to the effects on the plant. Equally, the absence of blue light in this treatment may have affected plant chemistry in some way, with knock-on effects for the aphid population, though the role of blue light in mediating plant defence is less well understood than that of UV or R:FR ratios. R:FR ratio presumably had no effect, as FR was absent, though the potential impact of the increased amount of red light on plant chemistry should not be completely discounted. Aphids did perform better under white light in this experiment, suggesting that pest management benefits may be obtained from crop production under red/blue LED regimes. Conversely, the experiment with two-spotted spider mite supported a pest suppressive effect under white light vs certain red/blue treatments. Though reasons for this remain unknown it can be speculated that spider mites were responding either to changes to plant physical structure (e.g. thickening of leaves) or chemistry, with visual acuity being more limited in this species (though spider mites display phototaxis and are believed to perceive UV/green).

Whilst the above results indicate that treatment effects on pest invertebrates can be expected under red/blue LED-based plant production, it deserves note that the experimental designs used have excluded any behavioural effects from being observed, as the target organisms have been placed directly on to the leaves of plants and enclosed either in small clip cages or bread bags to prevent migration. There are, however, many examples in the literature where light quality affects insect migration and location on a host plant. In the current project, the sticky trap data presented in the CP 125 year one report also indicate that landing site selection is disrupted under red:blue light treatments. Casual observations of pest species with the LED4CROPS facility also suggest that pest migration and flight are disrupted under the LED light environments. In particular pest migration appears to be

diminished, possibly because UV and bright light intensities are associated with flight initiation, and because insects are unable to identify a stimulus strong enough to drive phototaxis. Nevertheless, initial results collected to date support that parasitoid wasp flight activity is relatively unaffected by R/B light treatment, with relatively high activity (albeit reduced vs activity under R/B and white light) noted even under 100% red light. As 100% red light should be imperceptible to wasps (the majority of Hymenoptera having UV, blue and green photoreceptors), it could have been expected that minimal flight activity would occur under this treatment, as in the no-light control used. It is possible, however, that certain wavelengths of red light could trigger the wasps green photoreceptors, this perhaps being sufficient to initiate flight activity. Parasitism rates also appeared slightly reduced under 100% red light in one repeat of Experiment 4, potentially reflecting reduced wasp host-searching activity, though this was not consistent across runs of this experiment and parasitism *per se* was observed under all treatments.

It remains to be seen whether wasp orientation to an infested host plant is affected under different treatments, though this will be investigated later in the project. Should parasitoid wasps effectively navigate to infested plants, this would support their use under red/blue LED based plant production systems, with the data collected thus far evidencing both flight activity and (short-range) host location / parasitism as being relatively unaffected by the R/B light treatments used, albeit with potentially reduced activity under 100% red light. Amenability of predatory mites to use under R/B LED treatments will also be investigated, with initial casual observations (following general release within the LED unit) suggesting that good performance rates can be expected.

Conclusions

Results to date support that effects of red/blue LED-based plant production on pest and beneficial insects are highly variable and not necessarily transferable between pest species, crops, or combinations thereof. This is perhaps unsurprising given the range of plant and invertebrate responses that can be expected in response to varying light, and the multitude of ways in which light can affect both plants (e.g. alteration of physical attributes and chemical, including nutritional and plant defence, profiles) and invertebrates (e.g. disruption of circadian rhythm, visual host location, orientation upon a host plant, and physical and chemical host plant suitability). Disentangling drivers for specific invertebrate responses to given crop plants under LED production regimes will require a more detailed approach than can be assessed in the current project, though experiments to date do support that it may be possible, at least for certain pest-crop combinations, to design LED-based plant production systems that can be considered 'pest-suppressive' whilst still being amenable to biological control releases.

Knowledge and Technology Transfer

During this year (June 15 – May 16) we have received over 53 groups of visitors wishing to learn more about the LED facilities at STC. Groups range in size from single interested growers up to large organised groups with over 60 attendees. Demographically the visitors are diverse ranging from large grower organisation wishing to gain insight in to how they can implement LED technology in their business through to the horticultural college students learning about the technology for the first time. In addition Dr Davis has presented the results from CP 125 at several conferences and attended many other events to increase his knowledge base ensuring that this research remains at the forefront of this scientific field.

Presentations, Conferences and Other events

June 2015

Presented to the Harrogate Science group.

Presented at the AAB conference at Lancaster University.

Manned a stand at the Great Yorkshire Show that was demonstrating the uses of LEDs in horticulture.

September 2015

Presented at the TGA Conference

October 2015

Presented at the South West Growers Show.

Attended an IPPS Visit to Kernock Park Plants and helped answered questions regarding their use of LEDs.

Filming for CBeebies TV show that examined the use of LEDs for growing crops.

November 2015

Attended the Sainsbury's Farmer Conference.

January 2016

Presented at the AHDB Manipulation of spectrum for Horticulture conference.

May 2016

Attended the ISHS 8th Symposium on Light in Hort, East Lansing, MI, USA.

AHDB Grower articles

Colour co-ordinated pest monitoring. (Cover picture) December 2015/January 2016 Issue 219 pg. 12-14.

STC trial test scope for LED lighting. November 2015 issue 218 pg. 10.

LEDs: recipes to mix for edible crops. November 2015 issue 218 pg. 15-17.

LEDs: recipes to mix for ornamentals. October 2015 issue 217 pg. 16-18.

AHDB Technical guides

Lighting: The review (author)

Lighting: The principles (author)

Lighting: In practice (contributor)

Glossary

Cryptochrome	A photoreceptor that is sensitive to blue and UVA light.
Daily light integral (DLI)	A value of the total amount of light received over a 24 hour period. The values can be calculated using measurements made in different units. If irradiance (Wm^{-2}) values are used, the DLI has units of $\text{J m}^{-2} \text{d}^{-1}$. If photon-irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$) values are used, the DLI has units of $\text{mol m}^{-2} \text{d}^{-1}$.
Photon irradiance	A measurement of the number of photons incident on a surface, which has units of $\mu\text{mol m}^{-2} \text{s}^{-1}$.
Photoreceptor	Light-sensitive proteins that initiate light responses.
PAR	Photosynthetically active radiation (PAR) is light with wavelengths in the range of 400-700nm that can be used by plants for the process of photosynthesis.
Photomorphogenesis	The processes that causes plant morphology and pigmentation to change following exposure to light. These processes are activated and controlled by several photoreceptors.
Phototropin	A photoreceptor that detects blue and UVA light.
Phytochrome	A photoreceptor that can sense the red:far-red ratio of light.
UVR8	A photoreceptor that is able to detect UVB light.

References

- Bates, D., Maechler, M. & Bolker, B. (2012) *lme4: Linear mixed-effects models using Eigen and syntax*. R package version 0.999999-0.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, **24**: 127–135.
- Bowden, B. (1982) An analysis of factors affecting catches of insects in light-traps. *Bulletin of Entomological Research* **72**:535-556.
- Briggs, W.R. & Christie, J.M. (2002) Phototropins 1 & 2: versatile plant blue-light receptors. *TRENDS in plant Science* **7**:204-210.
- Colquhoun TA, Schwieterman ML, Gilbert JL, Jaworski EA, Langer KM, Jones CR, Rushing GV, Hunter TM, Olmstead J, Clark DG, Folta KM (2007) Light modulation of volatile organic compounds from Petunia flowers and select fruits. *Postharvest Biology and Technology* **86**: 37-44.
- Gardner, G. Lin, C. Tobin, E.M. Loehrer, H. & Brinkman, D. (2009) Photobiological properties of the inhibition of etiolated Arabidopsis seedling growth by ultraviolet-B irradiation. *Plant, Cell and Environment* **32**: 1573–1583
- Crawley, M.J. (2007) *The R Book*. 1st ed. John Wiley & Sons Ltd., Chichester, U.K.
- Fox, J. & Weisberg, S. (2011) *An R Companion to Applied Regression*. 2nd ed. Sage. Thousand Oaks, CA, USA.
- Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**: 346–363.
- Matteson N, Terry I, Ascoli-Christensen A & Gilbert C (1992) Spectral efficiency of the western flower thrips, *Franklinella occidentalis*. *Journal of Insect Physiology* **38**: 453-459.
- Mazza CA, Izaguirre MM, Curiale J & Ballaré CL (2009) A look into the invisible: ultraviolet-B sensitivity in an insect (*Caliothrips phaseoli*) revealed through a behavioural action spectrum. *Proceedings of the Royal Society* **277**:367-373.
- Nakamoto, Y. & Kuba, H. (2004) The effectiveness of a green light emitting diode (LED) trap at capturing the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) in a sweet potato field. *Applied Entomology and Zoology* **39**: 491-495.
- R Development Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria.
- Uran, O. Košvancová, M. Marek, M.V. & Lichtenthaler, H.K. (2007) Induction of photosynthesis and importance of limitations during the induction phase in sun and shade leaves of five ecologically contrasting tree species from the temperate zone. *Tree Physiology* **27**: 1207-1215.
- Weight, C. Parnham, D. Waites, R. (2008). LeafAnalyser: a computational method for rapid and large-scale analyses of leaf shape variation. *The Plant Journal* **53**: 578-586.
- Wu, H-C. & Lin, C-C. (2012) Red light-emitting diode light irradiation improves root and leaf formation in difficult-to-propagate *Protea cynaroides* L. plantlets in vitro. *Hortscience* **47**:1490–1494.
- Wyatt, I.J. & White, P.F. (1977) Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *Journal of Applied Ecology*, **14**: 757–766.